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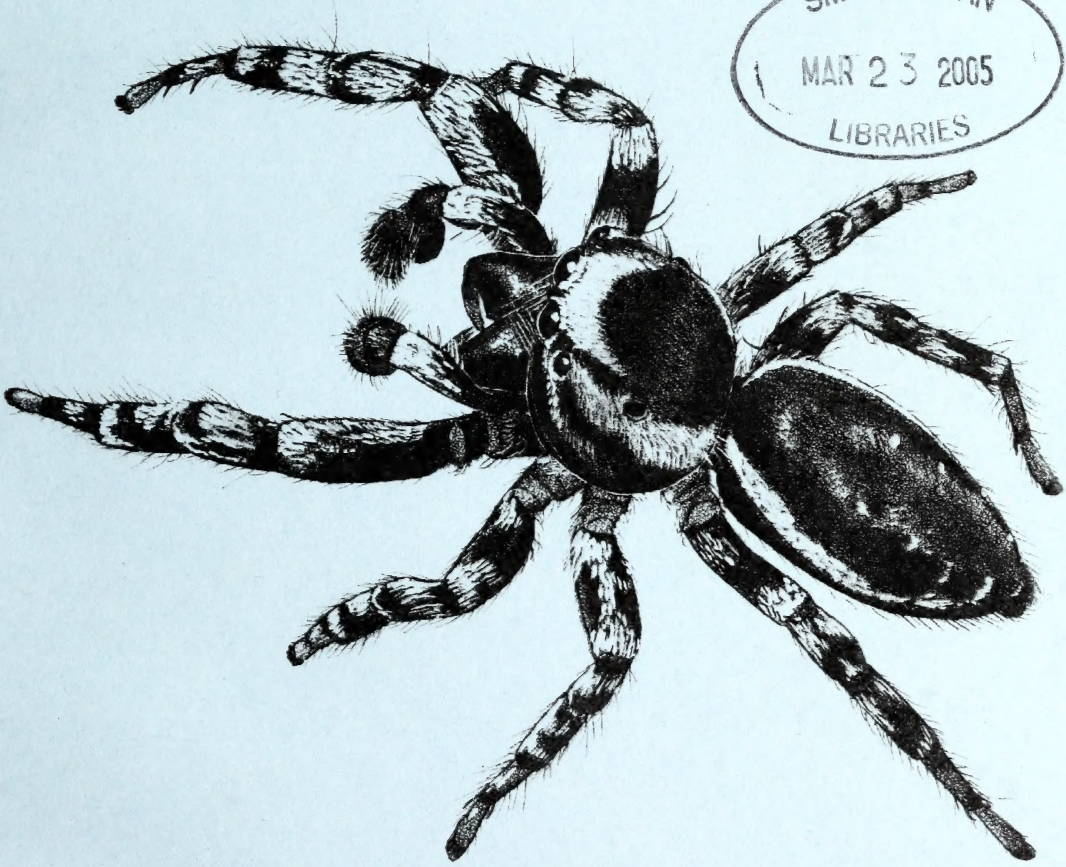

ESBC

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COVER: *Pelegrina montana* (Araneae: Salticidae)

Male of *Pelegrina montana* (Emerton, 1981), a salticid spider common throughout Canada and extending south into the northernmost and montane United States. Known from deciduous bushes and trees associated with streams, rivers and bogs.

Illustration on coquille board by pen, dark pencil and white paint.

Original art by Wayne Madison, courtesy of the Museum of Comparative Zoology, Harvard University.

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ERRATA

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Please note the following corrections to Volume 100 of the Journal of the Entomological Society of British Columbia:

Maclauchlan, L. E., L. Harder, J. H. Borden and J. E. Brooks. Impact of the western balsam bark beetle, *Dryocoetes confusus* Swain (Coleoptera: Scolytidae), at the Sicamous Creek research site, and the potential for semiochemical based management in alternative silvicultural systems. Journal of the Entomological Society of British Columbia 100:27-41.

1. Page 27, line 9: Leroy Harder address should read "206 – 330 East 1st Street, North Vancouver, B.C. V7L 1B5".
2. Page 39, line 42: Acknowledgements, change final sentence to read "This study was the subject of Leroy Harder's M.P.M thesis (Harder 1998) funded in part by Forest Renewal B.C. and the B.C. Forest Service."

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Simultaneous disruption of pheromone communication and mating in *Cydia pomonella, Choristoneura rosaceana* and *Pandemis limitata* Lepidoptera:Tortricidae) using Isomate-CM/LR in apple orchards

GARY J.R. JUDD[1] and MARK G.T. GARDINER[1]

ABSTRACT

Simultaneous disruption of pheromone communication and mating of codling moth, *Cydia pomonella* (L), and four leafroller (Lepidoptera:Tortricidae) species, *Choristoneura rosaceana* (Harris), *Pandemis limitata* (Robinson), *Archips rosanus* (L.) and *Archips argyrospilus* (Walker) using an incomplete mixture of their individual pheromone components was studied in organic apple orchards, in Cawston, BC, 1997. Multi-species disruption with a single 500 'rope' dispenser / ha application of Isomate-CM/LR was compared to a single 500 dispenser / ha application of Isomate-C. Season-long disruption was assessed using synthetic pheromone traps and laboratory-reared females in mating tables. Mean seasonal recaptures of sterile male *C. pomonella,* using 10 mg codlemone lures in orchards receiving releases of 1000 males / ha / week, were not significantly different in half-orchard plots (0.5 - 1 ha) of Isomate-CM/LR or Isomate-C. Mating of *C. pomonella* in Isomate-C- and Isomate-CM/LR-treated plots was negligible. Isomate-CM/LR significantly reduced catches of *C. rosaceana* and *P. limitata* relative to catches in Isomate-C-treated plots. Few *A. rosanus* and no *A. argyrospilus* were caught in any orchard. Mating of *C. rosaceana* and *P. limitata* in Isomate-CM/LR-treated plots was significantly less than in Isomate-C-treated plots. Our results indicate Isomate-CM/LR will disrupt mating of *C. pomonella* equivalent to Isomate-C and may provide sufficient disruption of leafrollers to supplement biological control in organic orchards. Further studies are needed to show impacts of mating disruption on leafroller populations and damage when applied to larger areas and for several seasons sequentially.

Key Words: Codling moth, leafrollers, mating disruption, Isomate, organic apples

INTRODUCTION

Disruption of pheromone communication and mating of moths by releasing synthetic pheromones into the atmosphere is being employed worldwide as a highly-specific alternative to insecticides (Jutsum and Gordon 1989; Ridgway *et al.* 1990; Cardé and Minks 1995). While the specificity of pheromone controls is appealing for integrated programmes, this specificity undermines use in crops where secondary pests may become primary pests when use of insecticides is reduced (Croft and Hoyt 1983). Apple production in the Pacific Northwest is a good example of where pheromone-based mating disruption (Howell 1992; Judd *et al.* 1996) as a replacement for broad-spectrum insecticides to control codling moth, *Cydia pomonella* (L.), has resulted in increasing damage from several species (Brunner *et al.* 1994; Knight 1995; Gut and Brunner 1998) that are controlled to some degree by insecticides targeting codling moth (Madsen and Proctor 1985).

One solution to this problem is to broaden the range of action of mating-

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disruption products by combining pheromone components common to more than one species, or by mixing uncommon pheromone components from different species. There are few published examples of multi-species mating-disruption (Ridgway *et al.* 1990; Cardé and Minks 1995) but where this has been attempted it usually involves pheromone components common to closely related species (Pfeiffer *et al.* 1993; Deland *et al.* 1994; Evenden *et al.* 1999a,b). For example, four species of leafrollers (Lepidoptera:Tortricidae) found infesting apple orchards in BC (Madsen and Madsen 1980), the obliquebanded leafroller, *Choristoneura rosaceana* (Harris), threelined leafroller, *Pandemis limitata* (Robinson), European leafroller, *Archips rosanus* (L.) and fruit-tree leafroller, *Archips argyrospilus* (Walker), all use (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac) as the major component in their multi-component pheromone blends (Arn *et al.* 1982). In small-plot studies, Deland *et al.* (1994) demonstrated that pheromone communication in *C. rosaceana*, *A. rosanus,* and *A. argyrospilus* could be disrupted with a 93:7 blend of *Z*11-14:Ac and (*E*)-11-tetradecenyl acetate (*E*11-14:Ac), and Evenden *et al.* (1999b) showed it was possible to simultaneously disrupt mating of *C. rosaceana* and *P. limitata* with a 98:2 blend of *Z*11-14:Ac and *E*11-14:Ac. Effectiveness of unattractive, generic pheromone blends as mating disruptants for these species is probably related to the fact that attraction to pheromone dispensers, i.e. false-trail following (*sensu* Bartell 1982), may not be necessary to achieve a high percentage of disruption (Knight *et al.*1998; Evenden *et al.* 1999a,b,c; Knight and Turner 1999).

The alternative idea of using one dispensing system to release pheromone components uncommon to all species being targeted has been around for some time (Deventer *et al.* 1992), but there are few examples or commercial products. Isomate-CM/LR is a multi-species mating-disruption product that was registered in 1997 for commercial use in the United States to control codling moth and leafrollers. Isomate-CM/LR was not registered in Canada at that time because efficacy data were lacking (Heather McBrien, personal communication) and to the best of our knowledge there are still no published data on its use in Canada. With a view towards registering mating-disruption products for simultaneous control of codling moth and leafrollers in BC we tested the utility of Isomate-CM/LR as a commercial mating-disruption product (Shin-etsu Chemical Company, Tokyo, Japan).

The primary objective of this study was to test the hypothesis that codling moth pheromone could be mixed in the same reservoir and released with pheromone components of leafrollers without compromising disruption of this 'key' pest of apples which can be controlled with registered single-species disruption products like Isomate-C (Judd *et al.* 1996,1997). Secondly, we wanted a season-long assessment of communication and mating disruption of leafrollers with Isomate-CM/LR in commercial orchards, because previous work was conducted for short test periods, in small plots, with noncommercial dispensers (Deland *et al.* 1994; Evenden *et al.* 1999a,b,c). Our third objective was to begin gathering efficacy data on Isomate-CM/LR for Canadian registration agencies which was lacking in 1997.

MATERIALS AND METHODS

Test Orchards. Experiments were conducted in five, commercial organic apple orchards located in the Similkameen Valley, Cawston, BC (Judd *et al.* 1997). Orchards ranged in size from 1 - 2 ha and were composed of mixed apple varieties planted at densities of 600 - 900 trees / ha with tree × row spacings of 2.4 - 4.6 × 4.6 - 5.5 m, respectively. Trees ranged in height from 2.5 - 3.5 m and were pruned using a pyramid shape training system. Wild populations of codling moth were very low

in these orchards due to successful use of mating disruption since 1990 (Judd *et al.* 1997) and benefits derived from release of sterile moths by the Okanagan-Kootenay Sterile Insect Release (SIR) programme (Dyck and Gardiner 1992). During the current study, SIR programme staff were releasing 1000 mixed sex (1:1 male:female ratio) sterile codling moths in each ha of every orchard two times each week. These standardized releases ensured there were approximately equal numbers of codling moths present in all orchards where we could assess disruption. No insecticides were applied to these orchards during this study.

Pheromone Disruption Treatments. Each orchard was divided into approximately equal halves (0.5 - 1 ha). One half was treated with Isomate-C and the other with Isomate-CM/LR. Both of these products are 'rope-type' twist-tie dispensers marketed by Pacific Biocontrol Corp., Vancouver, WA. Isomate-C is a translucent polyethylene dispenser containing a 155 mg blend of 58.8% (*E,E*)-8,10-dodecadien-1-ol (codlemone), 29.5% dodecanol, 5.3% tetradecanol and 6.4 % inert ingredients. Isomate-CM/LR is a brownish red polyethylene dispenser containing a 285 mg blend of 36.9% codlemone, 1.8% isomers of codlemone, 6.0% dodecanol, 1.2% tetradecanol, 43.5% *Z*11-14:Ac, 2.4% *E*11-14:Ac, and 8.2% inert ingredients, with similar design and release-rate characteristics as Isomate-C^{+} which has superceded Isomate-C as a commercial product but which contains the same active ingredients (Don Thomson, personal communication).

All pheromone dispensers were deployed at a rate of 500 / ha between 1 May and 6 May. Dispensers were attached to branches in the upper third of the tree canopy ca. 0.5 - 1.0 m below the tip of the central leader or on the first lateral branch down from the tip.

Disruption of Pheromone Communication. Disruption of pheromone communication in codling moth was assessed by comparing catches of SIR-released sterile male moths in synthetic pheromone-baited traps in the two disruption treatments. Two Pherocon 1-CP style closed wing traps (Phero Tech Inc., Delta, B.C.) baited with 10 mg of codlemone (99% isomeric and chemical purity, Shin-etsu, Fine Chemicals Division, Tokyo, Japan) were deployed in each pheromone-treated plot (two traps / half-orchard treatment). Traps were hung ca.1.5 - 2.0 m above ground near the centre of each treatment on 8 May and were checked weekly until 18 September. Trap bottoms were replaced weekly and lures were changed every third week. Sterile codling moths were identified by an internal red dye sequestered from the artificial diet used to rear them (Dyck and Gardiner 1992).

Trap catches in pheromone-treated orchards were compared with catches in adjacent, paired conventional orchards also receiving sterile moths. These insecticide-treated, but non-pheromone-treated orchards, are hereafter referred to as "non-treated". Two wing traps baited with 1 mg of codlemone were deployed as above in each non-treated orchard. Weekly catches in these orchards provide a seasonal record of sterile moth activity in the absence of pheromone-disruption treatments.

Disruption of pheromone communication in leafrollers was assessed by comparing catches of male *C. rosaceana*, *P. limitata*, *A. rosanus*, and *A. argyrospilus* in the two pheromone-treated halves of each organic orchard. Two Pherocon 1-C style open (5 cm spacer) wing traps (Phero Tech Inc., Delta, B.C.) baited with 3 mg (Deland *et al.* 1994) of each species' pheromone were deployed in the middle of each pheromone-treatment (two traps / species / disruption treatment × four species). One trap for each species was hung ca.1.5 - 2.0 m above ground on different sides of the same tree, in each of two separate trees, ca. 20 m apart in the centre row of each plot. Positions for all traps remained fixed throughout the season. Traps were checked weekly from June 6 until September 18 when moths were removed and counted. All pheromone lures were

changed at three-week intervals and trap bottoms were replaced as needed.

Synthetic pheromone baits for each leafroller species were prepared with chemical components (Aldrich Chemical Company Inc., Milwaukee, Wisconsin, USA) of known purity, as confirmed by gas-chromatographic analysis (*Z*11-14:Ac, 98% with 2% *E*11-14:Ac; *Z*11-14:Ald, 96%; *Z*11-14-:OH, 97%; Z9-14:Ac, 96%, and 12:Ac, 97%) using published ratios (Roelofs *et al.* 1976a,b; Vakenti *et al.* 1988; Deland *et al.* 1993).

In making pheromone lures for all five species, a 200 µl solution of each pheromone blend was dissolved in dichloromethane and loaded into separate red rubber septa (Aldrich Chemical Company Inc., Milwaukee, Wisconsin, USA). After loading, septa were air dried for ca. 18 h at 23 °C in a fume hood and stored at 0 °C until pinned to the inner side of trap lids in the field.

Disruption of Mating. Disruption of mating was assessed using virgin female moths in mating tables described by McBrien and Judd (1996). Only mating of *C. pomonella*, *C. rosaceana*, and *P. limitata*, was assessed because these were the only species we had in rearing. *C. pomonella* were reared on an artificial diet (Dyck and Gardiner 1992) at 27 °C under a 16:8 h L:D photoregime and *C. rosaceana* and *P. limitata* were reared on a modified pinto bean-based diet (Shorey and Hale 1965) at 24 °C and 16:8 h photoregime. Female pupae of each species were placed individually in 150-ml plastic cups provided with a wet cotton wick until adults eclosed. Female moths aged 24 - 72 h were immobilized at 0.5 °C and one forewing and a tarsal tip were clipped with fine forceps before transporting them to field sites in refrigerated containers.

Individual females of these three species were placed in the same tree, in the upper third of the canopy, in each of five trees laid out in a die pattern centred in each pheromone-treated plot. Females were several trees removed from their respective species-specific pheromone traps. Availability of female *C. pomonella* allowed us to deploy mating tables in non-treated orchards for comparison, but shortages of leafrollers prevented this deployment. All female moths were placed in the field in the afternoon and removed the following morning to minimize predation and escape. Females recovered from the field were returned to the laboratory and each *bursa copulatrix* was dissected and examined for the presence of a spermatophore which indicates they had mated. Females were omitted from the data if they were dead when recovered.

Female *C. pomonella* were placed in the field on two nights, every second week from 19 May until 6 September. Female leafrollers were placed in the field on 2 to 4 nights, every week for three consecutive weeks starting 3 July and 12 August which corresponded to peak flight periods of first and second generation, respectively.

Harvest Fruit Damage. Although our experiments were not designed to evaluate crop protection specifically, a sample of 2500 fruit was taken from each pheromone-treated plot in each of the five organic orchards in order to establish background levels of leafroller damage for future reference. Samples were taken during normal harvest dates as fruit maturity and growers dictated. All fruit were examined for damage from codling moth, including stings and deep entries, and early and late season leafroller feeding. Each half-orchard treatment was sampled by walking a "W" pattern from corner to corner and edge to edge and systematically choosing 25 trees, 5 edge and 20 interior trees. One hundred fruit were removed from each sample tree by picking 50 low and 50 high fruit from south-side branches.

Statistical Analyses. For each species, moth captures from both traps in each pheromone treatment in the same orchard were pooled and transformed ($\log_{10}[x+1]$) to normalize the data. Mean seasonal cumulative moth catches in the two pheromone treatments were compared using an analysis of variance (ANOVA) appropriate for a randomized block design (Zar 1984),

where orchards are blocks (n = 5) and there are 2 pheromone treatments assigned randomly to either half orchard. Statistical comparisons were not made between codling moth catches in pheromone-treated and non-treated orchards because the latter orchards were treated with insecticides that could confound any comparison. In addition, different strength lures were used to monitor codling moth in pheromone-treated and non-treated orchards. However, catches of sterile codling moths from non-treated orchards are presented for comparisons of their relative seasonal activity. Low recovery of females in mating tables meant mean percentage mating per orchard could not be estimated reliably, therefore recovered females were pooled across orchards by treatment and χ^2 tests were used to compare the frequency of mating in the two pheromone treatments. A randomized block ANOVA was used to compare mean damage estimates from the two treatments after an arcsine $\sqrt{p}$ transformation of the data.

RESULTS AND DISCUSSION

Disruption of Pheromone Communication in *C. pomonella*. In non-treated orchards the mean (± SE) seasonal catch of sterile codling moths / trap (1051.7 ± 307.2) was several times greater than catches in either pheromone-treatment (Table 1). Comparing catches of *C. pomonella* in this way likely underestimates the level of disruption achieved by pheromone treatments, because traps were baited with different strength lures and insecticides may have reduced catches of sterile moths in non-treated orchards. While the SIR programme dictated use of 1 mg lures in conventional orchards, we chose to use 10 mg lures in pheromone-treated plots because they ensured sufficient moth captures to detect differences in pheromone treatments. Disorientation of *C. pomonella* would likely appear greater had we used 1 mg lures in pheromone-treated orchards, because they catch very few moths in this situation relative to 10 mg lures (Judd *et al.* 1996). Correcting for this difference in relative attraction we calculate from catches in Table 1, that Isomate-C caused 91% and Isomate-CM/LR caused 90% disorientation relative to the 1051 moths caught / trap in non-treated orchards.

There was no significant difference ($P > 0.05$) in the mean seasonal number of sterile moths caught / trap in plots treated with Isomate-C (274.5 ± 44.4) or Isomate-CM/LR (311.9 ± 126.2). This result indicates that both pheromone treatments disrupted orientation of codling moth to a similar extent and therefore, the release of leafroller pheromone components from Isomate-CM/LR had no detrimental affect on disruption of codling moth pheromone communication (Table 1).

Mean weekly catches of sterile *C. pomonella* pooled across pheromone-treated orchards (Fig. 1A) reveals few weekly differences between rates of recapture in plots treated with Isomate-C or Isomate-CM/LR, suggesting both dispensers were equally disruptive under varied weekly weather conditions. Weekly catches of sterile moths under both pheromone treatments was low early in the season and began to increase near 3 July (Fig. 1A). This increase is more likely caused by a general increase in the activity of sterile moths, or response to traps as the season progressed, than by decreasing effects of disruption treatments. This conclusion is based on the fact that catches of sterile males in non-treated orchards also doubled in weeks 9 - 16 compared with weeks 1 - 8 (Fig. 1A).

Disruption of Pheromone Communication in Leafrollers. Mean seasonal catches of *C. rosaceana* and *P. limitata* were both significantly ($P < 0.05$) lower in the Isomate-CM/LR treatment than in the Isomate-C treatment (Table 1). Almost no *Archips spp.* were caught in any orchards, supporting the view that these species are generally found further north and more

Table 1.
Seasonal cumulative catches of male moths in synthetic pheromone-baited traps and mating of sentinel females in mating tables for each of four species in each half of five organic apple orchards treated with different commercial pheromone dispensers, Cawston, BC, 1997.

Moth species[1]	Commercial pheromone disruption treatment (500 dispensers / ha)	Mean (± SE) number of moths / trap[2]	% of females mating[3]
C. pomonella	Isomate-C	275.5 ± 44.4a	0.0a
	Isomate-CM/LR	311.9 ± 126.2a	0.9a
C. rosaceana	Isomate-C	752.7 ± 356.8a	25.9a
	Isomate-CM/LR	124.2 ± 187.6b	7.8b
P. limitata	Isomate-C	542.7 ± 241.5a	32.6a
	Isomate-CM/LR	84.7 ± 103.7b	5.6b
A. rosanus	Isomate-C	2.0 ± 0.8 nt	0.0 nt
	Isomate-CM/LR	0.0 ± 0.0	0.0
A. argyrospilus	Isomate-C	0.0 ± 0.0 nt	0.0 nt
	Isomate-CM/LR	0.0 ± 0.0	0.0

[1] All *C. pomonella* caught were sterile moths released (1000 males / ha / orchard / week) by the Okanagan-Kootenay Sterile Insect Release Programme.
[2] Means of seasonal cumulative catches in two traps in each of five orchards. Paired means within a species followed by different letters are significantly different ($P < 0.05$) by Randomized Block ANOVA. nt = no test.
[3] Paired percentages within a species followed by different letters are significantly ($P < 0.05$) different by χ^2 tests on observed frequencies of mating.

frequently in the Okanagan than in the Similkameen Valley (Madsen and Madsen 1980).

Weekly catches of *C. rosaceana* are shown in Fig. 1B. Catch curves in both treatments reflect a large first-generation and small second-generation flight. This pattern is typically seen in insecticide-treated orchards and is thought to occur because insecticides applied during first generation reduce second-generation populations (Madsen and Madsen 1980). This cannot explain our results because organic orchards were not treated with insecticides. A small second-generation flight may arise because first-brood larvae often enter diapause early in this region. Therefore, control of this first generation is important because it prevents an escape of insects that give rise to an overwintering population with potential to undermine long-term control.

Weekly catches of *C. rosaceana* were reduced 70.9 - 100% in the Isomate–CM/LR treatment relative to the Isomate-C treatment (Fig. 1B), and reductions averaged 90.3% for the season across all orchards (Table 1). Deland *et al.* (1994) reported a similar 89 - 91% average disorientation of *C. rosaceana* to synthetic pheromone traps in 0.1 ha plots treated with 1000 - 2000 Hamaki-con (Shin-etsu Chemical Company Ltd., Tokyo, Japan) dispensers per ha, releasing a 93:7 blend of *Z*11-14:Ac and *E*11-14:Ac at ca. 20 - 40 mg / ha / h (Deland 1992). Given that a single Isomate-CM/LR dispenser releases ca. 1 mg *Z*11-14:Ac / day in the Okanagan Valley (GJRJ, unpublished data), 500 Isomate-CM/LR dispensers would release ca. 21 mg / ha / h. Therefore, using more than 500 Isomate-CM/LR dispensers per ha to disrupt *C. rosaceana* would seem unwarranted.

Weekly catches of *P. limitata* are shown in Fig. 1C. Unlike catches of *C. rosaceana*, which showed two distinct peaks in the Isomate-C treatment (Fig. 1B), catches of *P. limitata* remained high all season in the Isomate-C treatment (Fig.

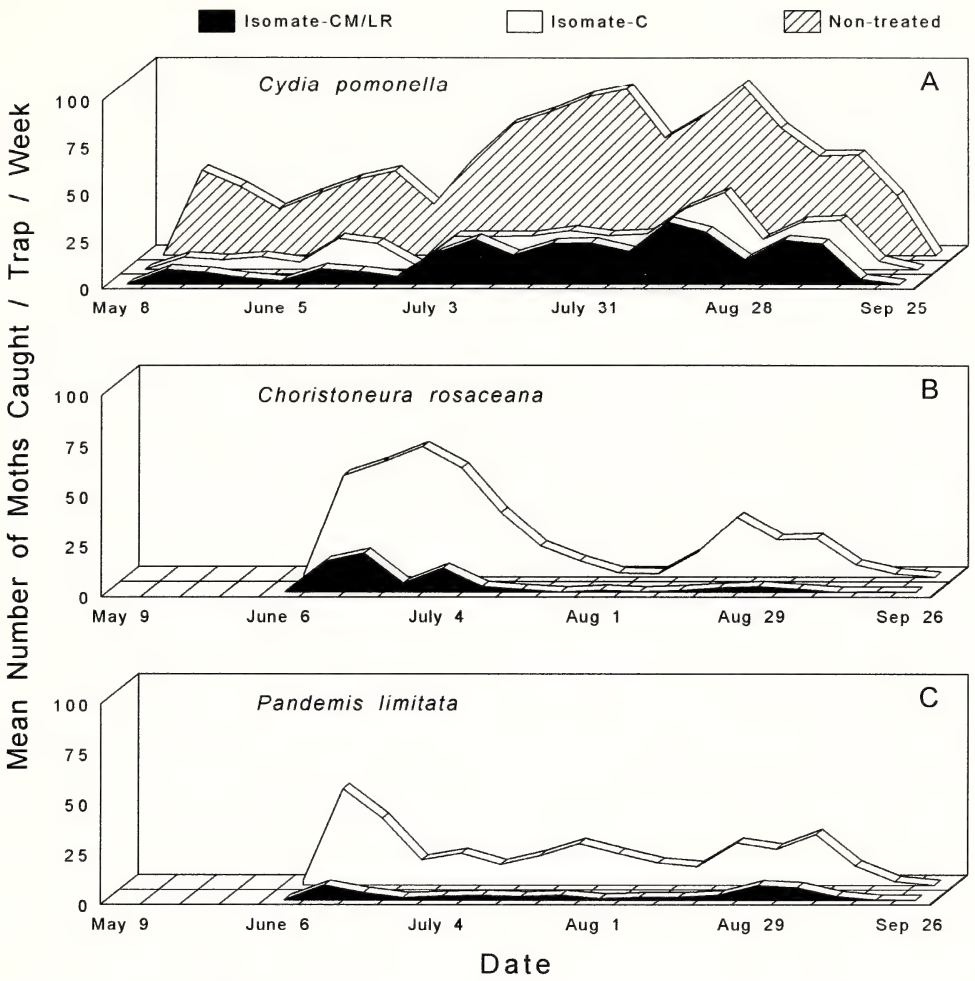

Figure 1. Mean weekly catches of mass-reared, sterile male *Cydia pomonella* released by the Okanagan-Kootenay SIR Programme (**A**), wild male *Choristoneura rosaceana* (**B**) and wild male *Pandemis limitata* (**C**) in their respective species-specific synthetic pheromone-baited traps hung in non-treated conventional apple orchards (*C. pomonella*) and in Isomate-C- and Isomate-CM/LR-treated halves of organic apple orchards (all species) in Cawston, BC, 1997.

1C). This is quite different from the two distinct peaks Madsen and Madsen (1980) observed in insecticide-treated conventional orchards. Catches of *P. limitata* in the Isomate-CM/LR treatment showed early and late season peaks, but moths were always being caught in low numbers. A second peak in the Isomate-CM/LR treatment may indicate leafroller pheromone was running out late in the season as found by Knight *et al.* (2001). Given the date dispensers were deployed and a 1 mg / day release rate, dispensers were expected to run out of *Z*11-14:Ac between 24 - 30 August, exactly when the second peak of catches occurred (Fig. 1C). To make it a reliable product more data are needed on the release of various pheromone components from Isomate-CM/LR dispensers in relation to temperature.

Weekly catches of *P. limitata* were reduced 67 - 100% by treatment with Isomate-CM/LR (Fig. 1C) and reductions averaged 88.9% for the season across all orchards (Table 1), relative to the Isomate-C treatment.

Disruption of Mating in *C. pomonella*. Mating of *C. pomonella* in non-

treated orchards receiving sterile moths averaged 48.7% during late May and June and 59% in July and August. In spite of very large numbers of sterile males being released in this study (1000 / ha / week), almost no sentinel female *C. pomonella* mated in either pheromone treatment, and there was no significant difference (χ^2 = 0.34, df = 1, $P > 0.05$) in the frequency of mating in the Isomate-C (0 / 223) and Isomate-CM/LR treatments (2 / 212). Mating frequency of *C. pomonella* in our Isomate-C treatment was quite low when compared with the frequencies of mating among feral codling moths reported by Knight (1996) using passive pane traps, or by Howell (1992) using black light traps, both in Isomate-C-treated orchards. While there may be discrepancy between different assessment methods, our evaluation of mating is suitable in this context because the relative difference in mating between the two pheromone treatments is of interest here. Given that Isomate-C is known to control populations of *C. pomonella* (Judd *et al.* 1996) and there appears to be little difference in levels of trap disorientation and mating disruption using Isomate-CM/LR, we expect the level of control with these two products to be similar.

Disruption of Mating in Leafrollers. On average, 25.9% (14 / 54) of female *C. rosaceana* and 32.6% (34 / 104) of female *P. limitata* mated when placed in the Isomate-C treatment having no leafroller pheromone, while only 7.8% (5 / 64) and 5.6% (6 / 107) of each species, respectively, mated in the Isomate-CM/LR treatment. Isomate-CM/LR significantly (χ^2 = 5.84, df = 1, $P < 0.05$) reduced mating of *C. rosaceana* by 70% and *P. limitata* by 83% (χ^2 = 23.4, df = 1, $P < 0.001$), relative to the Isomate-C treatment. Our seasonal level of mating disruption in *C. rosaceana* was somewhat lower than the 86% observed by Evenden *et al.* (1999b) using a similar blend (100:2 ratio of *Z*11-14:Ac and *E*11-14:Ac) and the same dispenser density (500 / ha) in 0.1 ha plots. Lower levels of disruption in our study may be due to higher population densities, or movement of *Z*11-14:Ac into the Isomate-C treatment used for comparison. Simultaneous release of codlemone from Isomate-CM/LR dispensers cannot explain any differences between these studies because *C. rosaceana* is not known to detect this chemical (GJRJ, unpublished electroantennograms).

Mating of *C. rosaceana* was similar in first (7.4%) and second generation (8.1%) and although catches of *P. limitata* increased during second generation (Fig. 1C), there was no increase in the frequency of their mating between first (7.5%) and second (2.4%) generation in Isomate-CM/LR-treated plots. Therefore, the amount of pheromone being released late in the season appears to have been adequate to control mating in leafrollers even if catches appeared to increase (Fig. 1C). Our 83% seasonal average mating disruption for *P. limitata* was similar to the 85% observed by Evenden *et al.* (1999b) when 1000 dispensers / ha were employed. Like *C. rosaceana* above, there may be little value in using more than 500 Isomate-CM/LR dispensers per ha to disrupt mating in *P. limitata*.

Fruit Damage. As expected given low levels of wild codling moths, release of sterile males, and treatment with pheromone, there was no detectable codling moth damage in any orchard. Leafroller damage was very high in these organic orchards and there was no significant ($P > 0.05$) difference in the mean percentage of damage in plots receiving Isomate-C (10.9 ± 5.4%) or Isomate-CM/LR (8.7 ± 4.7%). These damage levels suggest densities of leafrollers were very high and crop protection can fail under high population densities even if measures of disruption are large (Judd *et al.* 1996, 1997). While we do not consider our experiment to be a fair evaluation of leafroller control by mating disruption, these data should provide a useful baseline for longer-term mating-disruption trials.

Our studies have demonstrated it is possible to simultaneously disrupt pheromone communication and mating in sym-

patric tortricid moths by releasing an incomplete mixture of their individual pheromone components. Levels of disorientation and reductions in mating of all species with Isomate-CM/LR were comparable to those seen in studies examining each species individually (Deland *et al.* 1994; Evenden *et al.* 1999a,b,c). Our data suggest control of codling moth with Isomate-CM/LR should be comparable to control with registered products like Isomate-C^{+} and Isomate-C (Judd *et al.* 1996). Isomate-CM/LR also has potential for supplementary control of leafrollers. These conclusions are supported by several studies using Isomate-CM/LR in Washington State (Knight *et al.* 1997; Knight 1998; Knight *et al.* 2001). These non-refereed reports suggest that when used on an area-wide basis for three years (1998 - 2000), orchards treated with Isomate-CM/LR and supplemental insecticides had 41% less leafroller damage, received one less spray per season and consistently had less codling moth damage than orchards receiving Isomate-C^{+} and supplemental sprays.

Lacking in these studies unfortunately, are data from Isomate-CM/LR-treated orchards receiving no supplemental insecticides. Ours is the only evaluation of Isomate-CM/LR where no insecticides were applied and the only one reporting actual mating data that we are aware of. These kinds of data are useful for registration of pheromones in Canada. Efficacy data based on fruit damage are also needed but our study lacks this assessment because the split-orchard design resulted in treatment areas too small to prevent movement of mated females or ballooning larvae between treatments. Therefore, it remains to be shown whether measures of disruption observed here will impact the population dynamics of these species and control damage effectively without use of insecticides. In this regard, Isomate-CM/LR may have a role to play in supplementing biological control of leafrollers in organic apple orchards where parasitism rates as high as 68% are common during summer in the Similkameen Valley (Cossentine *et al.* 2004).

During the revision of this manuscript Isomate-CM/LR was registered by the Canadian Pest Management Regulatory Agency (Heather McBrien, personal communication). Nevertheless, multiple-year mating-disruption trials still need to be conducted in larger orchards, or on an area-wide basis (Knight *et al.* 2001), and under lower population pressure than seen in this study, before any conclusions about the efficacy of mating disruption as a crop-protection tool for leafrollers in BC can be made.

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Emergence of overwintered larvae of eye-spotted bud moth, *Spilonota ocellana* (Lepidoptera: Tortricidae) in relation to temperature and apple tree phenology at Summerland, British Columbia

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ABSTRACT

We recorded daily appearance of overwintered larvae of eye-spotted bud moth (ESBM), *Spilonota ocellana* (Denis & Schiffermüller) in spring 1992, 1994, and 1996 in an unsprayed apple orchard at Summerland, British Columbia, to relate larval emergence to degree-day (DD) accumulation and apple phenology. In all years the first larva was found between mid-March and early April, and none appeared after late April. Median emergence of larvae occurred when McIntosh apple trees were at early, tight-cluster stage of fruit-bud development. Larval head capsule measurements showed that ESBM usually overwinter as fifth and sixth instars, with a small proportion (≤6%) as fourth-instar larvae. In the laboratory we monitored emergence of non-diapausing overwintered larvae from apple branches incubated at 8.8, 9.4, 12.9, 15.0, 18.0, and 20.9 °C. A least-squares linear regression described emergence over this temperature range relatively accurately ($r^2 = 0.57$, $P < 0.05$) and a base temperature for emergence (T_b = 1.0 °C ± 0.6) was extrapolated from this regression. Regression analysis indicated median emergence should require 154.6 ± 6.7 DD above 1 °C ($DD_{1\,°C}$). Using daily air-temperature maxima and minima and 1 March to start accumulating $DD_{1\,°C}$, the error between predicted and observed days to median emergence in the field was -6.7 ± 3.1 d; the regression model predicted early in every case. Using observed larval appearance on apples (1992, 1994, & 1996) and an iterative process, we determined that a combination of 6 °C as the T_b and 1 January as a date to start accumulating $DD_{6\,°C}$, minimized the coefficient of variation for the three-year mean $DD_{6\,°C}$ accumulations (82.7 ± 3.5 $DD_{6\,°C}$) required for 50% of the larvae to appear in the field. While this latter DD index described observed emergence of larvae accurately, and its use may help improve management of ESBM, it should be validated using independent data before growers use it routinely.

Key Words: *Spilonota ocellana*, Tortricidae, larval development, phenology, degree days

INTRODUCTION

The eye-spotted bud moth (ESBM), *Spilonota ocellana* (Denis and Schiffermüller), is a pest of apple (Gilliatt 1932, MacLellan 1978), blueberry (Gillespie 1985), cherry (Oatman *et al.* 1962), and prune (Madsen and Borden 1949) throughout fruit-growing areas in the northern hemisphere (Weires and Riedl 1991). ESBM is univoltine and larvae overwinter in hibernaculae on branches of host plants. Larvae crawl from hibernaculae in early spring to feed on leaves and blossoms. Pupation occurs in a nest of dead leaves and blossoms held together with silk.

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Adults emerge in early summer and lay eggs singly on leaves (Weires and Riedl 1991, McBrien and Judd 1998). Summer-generation larvae arising from these eggs often feed on fruit surfaces causing damage and discolouration beneath leaves attached to fruit with silk (Gilliatt 1932).

In North America, ESBM has a history of sporadic outbreaks in apple orchards (MacLellan 1978) because insecticides applied in summer against codling moth, *Cydia pomonella* (L.) often control it indirectly (Madsen and Downing 1968, British Columbia Ministry of Agriculture, Fisheries and Food 2004). As non-insecticidal methods like sterile male technique (Dyck and Gardiner 1992) or pheromone-based mating disruption (Judd *et al.* 1996, Judd and Gardiner 2004) have been implemented to control codling moth and leafrollers (Tortricidae) in British Columbia (B.C.), feeding damage by ESBM has increased (GJRJ unpublished data), mirroring reports from The Netherlands (Deventer *et al.* 1992). Therefore, control of ESBM in spring has become more critical. Insecticides applied in spring are often timed to control leafrollers and green fruit worms (Noctuidae), providing control of ESBM only indirectly (Madsen and Downing 1968, British Columbia Ministry of Agriculture, Fisheries and Food 2004). Therefore, strategies to control ESBM specifically need to be developed.

The ability to predict when overwintered larvae of ESBM appear in spring would be a useful tool in designing an integrated management programme. The phenology of ESBM larval emergence in spring has been related to apple phenology in other areas (Gilliatt 1932, Madsen and Borden 1949, Oatman *et al.* 1962) but this approach has not been validated in B.C., Canada, and may not provide consistent prediction of emergence on different species of host plant and on different varieties of fruit trees across different years. A temperature-based model to predict emergence of overwintered larvae may be a more useful approach as this technique has been applied successfully against other species of leafrollers in the Pacific Northwest (Brunner 1991). We describe emergence of overwintered larvae of the ESBM in relation to degree-day (DD) accumulations and apple tree phenology.

MATERIALS AND METHODS

Collecting and Handling Prunings. Several hundred 30-cm branch sections were pruned from a mixed block of McIntosh, Delicious, and Spartan apple trees in an experimental apple orchard at the Pacific Agri-Food Research Centre (PARC) in Summerland, B.C. on 2 February 1992. No insecticides were applied to this orchard for at least five years preceding or during this study and it was heavily infested with ESBM larvae in 1991. Prunings consisting mainly of fruit-spur wood and excluding previous years' growth were transported to the laboratory, stored in cardboard boxes filled with moist sawdust, and held in darkness at 0.4 ± 0.5 °C until required.

Diapause Termination. Before assessing temperature-dependent emergence of overwintered larvae, it was important to ensure they had completed diapause so that some portion of diapause development was not included in any estimates of post-diapause development time. Prunings collected on 2 February and 1 March 1992, when apple buds were still dormant, were placed in a controlled-environment chamber at 19 °C under a 13:11 h L:D photoregime provided by Daylight fluorescent tubes. On each collection date, seventy 30-cm-long prunings were placed in a plastic basin (35 cm × 35 cm × 16 cm), covered with polyester organza and held in place with an elastic band that prevented larva from escaping but permitted air circulation. On 1 March, an equivalent length of pruned branch sections was removed from laboratory cold storage (0.4 °C) and set up identical to other pruning samples. Every 24 h, prunings were re-

moved from their basin and tapped sharply to dislodge active larvae onto a white cloth. The number of larvae collected daily was recorded and sampling was terminated when larvae went undetected for seven consecutive days after they began appearing.

Emergence at Constant Temperatures. On 7 May 1992, 66 days after being placed at 0.4 °C as part of the diapause study, ca. five hundred 30-cm sections of prunings were removed from cold and divided evenly among eight basins described previously. One basin of prunings was placed in each of seven separate controlled-environment chambers set at 3.8, 8.8, 9.4, 12.9, 15.0, 18.0, or 20.9 °C, respectively, each with a photoregime of 13:11 h L:D. One basin of prunings was returned to 0.4 °C. Constant-temperature conditions were chosen to approximate the range of air temperatures and photoregime normally experienced by ESBM larvae during spring in the Okanagan Valley. When prunings were placed in controlled-temperature chambers on 7 May 1992, larvae in the field had completed emergence.

One larva emerged on day 54 from prunings incubated at 0.4 °C and three larvae emerged on days 9, 18, and 46 from prunings incubated at 3.8 °C. Therefore, on 1 July, 55 d after incubation, prunings were transferred from 0.4 and 3.8 °C to the 18 °C constant-temperature incubator to determine if larvae would emerge at the higher temperature and if their emergence times would be shorter than those placed at constant 18 °C from the outset.

Linear Regression-based Emergence Model. A linear DD emergence model, lower threshold base temperature (T_b), and the DD requirements for median (50%) emergence of larvae were determined analytically using linear regression techniques (Campbell *et al.* 1974) applied to laboratory-derived constant-temperature emergence data. Emergence time in days for each larva at each constant temperature was converted to an emergence rate by taking the reciprocal (developmental rate = 1 / days to emerge). Emergence rates for all larvae at each temperature were regressed against temperature using least-squares linear regression analysis (Zar 1984). Extrapolating this linear regression through the *x*-axis gave the theoretical lower developmental threshold base temperature (Arnold 1959). The number of DDs needed for emergence of 50% of the larval population was determined by taking the reciprocal of the slope of the linear regression line (Campbell *et al.* 1974). Standard errors for estimates of T_b and DD totals were calculated as described by Campbell *et al.* (1974). Two ESBM larvae emerged within 1 d of incubation at 20.9 °C. The emergence rates for these two larvae were considered outliers and excluded from linear regression analysis in order to maintain homogeneity of variance (Zar 1984).

Phenology of Larval Emergence in the Field. Prunings infested with overwintered ESBM larvae were collected from the experimental apple orchard in early March 1992, 1994, and 1996. Collections were made while apple buds were dormant and before ESBM larvae had started to crawl from overwintering hibernaculae. As before, prunings mainly consisted of fruit-spur wood and did not include previous years' growth. Wood was cut into 30-cm-length pieces and placed in cylindrical mesh bags (length = 60 cm, diameter = 25 cm) made from polyester organza and tied at both ends with string. Each of eight mesh bags was filled with 30 prunings and suspended 1.8 m above ground from an apple tree in the experimental orchard. Bags were suspended from a branch on the north side of trees to minimize exposure to direct sunlight. Each bag was hung from a separate tree in the lower half of the canopy but above the lowest scaffold branch. No bags were hung in the border row of trees.

Each day, each pruning was removed from its bag and tapped sharply on a white plastic tray to dislodge active larvae. The inside of each bag was checked for larvae after which prunings were returned to bags

and rehung in trees. Numbers of larvae collected from each bag were recorded, and the total number collected from all bags was pooled each day. Bags were checked until no larvae were counted for seven consecutive days after the first larva appeared. The phenology of a sample of 20 fruit buds on McIntosh apple trees in the study site was recorded every two to three days. Fruit-bud phenology is described by British Columbia Ministry of Agriculture, Fisheries, and Food (2004).

Instars of Overwintered Larvae. Head capsule widths of overwintered larvae were measured from prunings collected on 1 March 1992 as part of the diapause termination study, and from prunings used to monitor larval emergence in the field during 1994 and 1996. Each larva that emerged from prunings was preserved in 70% ethanol and its head capsule width was measured to determine instar (Gilliatt 1932). Gilliatt (1932) did not provide estimates of variation for the head capsule width of each of the seven larval instars, therefore, the midpoint between the mean head capsule width for each successive instar was used as the range for classifying each instar.

Temperature Measurement and DD Accumulation. Hourly air temperatures in the PARC apple orchard were recorded throughout the study using a DP-212 datapod (Omnidata™, Logan, UT, USA) housed at 1-m height in a Stevenson screen. Degree-day summations were calculated by fitting a sine wave (Allen 1976, case 4) to daily air-temperature minima and maxima using the computer programme described by Higley *et al.* (1986). Degree-day summations were calculated in 1992, 1994 and 1996 using all possible combinations of lower threshold base temperatures consisting of 1, 2, 3, 4, 5, 6, 7 or 8 °C, and 1 January, February, or March as dates to start summation. Accumulations from each start date up to the Julian Day (JD) on which 50% of the larvae appeared in the phenology sampling performed above were calculated. Temperatures <9 °C were chosen as possible base temperatures because the majority of larvae incubated at constant temperatures <8.8 °C in the laboratory never emerged. No upper temperature threshold was set. The coefficient of variation (CV) among these DD calculations for individual years was calculated for each combination of base temperature and starting date. The combination of base temperature and starting date which gave the lowest CV (Arnold 1959) was chosen to generate DD summations that best described the observed cumulative larval emergence in 1992, 1994 and 1996. Cumulative larval emergence data (1992, 1994, and 1996) was plotted against these DD summations and a cumulative Weibull function was fitted to this scatter plot (McBrien and Judd 1998).

Statistical Analyses. All statistical analyses were performed with SigmaStat® (Version 3.0.1, SYSTAT Software Inc., Richmond, California, USA) and all experiment-wise error rates were set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Diapause Termination. The average number of days required for ESBM larvae to appear from prunings incubated at 19 °C following collection at various dates or removal from cold storage are shown in Table 1. Fifty percent of the 74 larvae coming from prunings collected on 2 February appeared in 9.7 d, whereas 50% of the 66 larvae coming from prunings collected on 1 March did so in 6 d. This difference represents a 38.1% decline in days to appear following 28 d of field aging. By comparison, 50% of the 83 larvae coming from prunings collected on 2 February but stored at 0.4 °C for this 28-day period, did so in 8.6 d, representing an 11% decline in days to appear compared with larvae on prunings collected 2 February, but immediately incubated at 19 °C (Table 1).

Knowing when diapause terminates is critical to construction and application of any DD model because it establishes a

Table 1.
Time needed to emerge (days) at 19 °C for larvae of *S. ocellana* collected from the field or removed from laboratory cold storage in February and March 1992.

Collection and incubation dates	Median emergence time		Mean (± SD) emergence time	
	Laboratory stored[1]	Field collected	Laboratory stored[1]	Field collected
2 February	-	9.7	-	10.8 ± 2.3
1 March	8.6	6.0	9.0 ± 2.3	7.0 ± 2.3

[1] One half of first field collection (2 February) was placed in laboratory cold storage (0.4 °C) and removed at time of second field collection (1 March)

biologically relevant time at which these accumulations might start and it ensures more accurate determination of post-diapause development times. Whether overwintering larvae of ESBM undergo true obligatory diapause (Danks 1987) is not known nor was it determined because prunings were collected too late in winter to investigate this aspect of larval development. Those cues that might end diapause in ESBM are not known, but in temperate insects there is often a minium time requirement before post-diapause development begins (Danks 1987). Whatever cues are necessary to break diapause in ESBM, larvae clearly develop in February if temperatures are appropriate. As diapause progresses or terminates in many species, observed times for the diapausing life stage to hatch or emerge often shortens and becomes more synchronous (Tauber and Tauber 1976). Thus, a decline in average days to appear or emerge during incubation that begins at different times in winter is often an outward manifestation of diapause progression (Judd *et al.* 1993, 1994). Our observation that average time until larvae appeared on prunings incubated at 19 °C after storage at 0.4 °C from 2 February to 1 March, was only 11% shorter than those incubated at 19 °C on 2 February (Table 1), suggests most larvae were out of diapause by 2 February. By comparison, larvae stored in the field during this same period appeared sooner when placed at 19 °C on 2 March (Table 1) than on 2 February, indicating they had probably undergone some post-diapause development in the field. Therefore, most ESBM larvae probably complete diapause by 2 February and undergo post-diapause development when temperatures are above 0.4 °C as they were in the field at this time of year. Collectively, these data indicate larvae on prunings removed from cold storage after 1 March were suitable for studies on post-diapause development and 1 February might be a suitable time to start DD accumulations in the field.

Emergence at Constant Temperatures. The relative frequency of days to appear for larvae incubated at constant temperatures between 8.8 and 20.9 °C are shown in Fig. 1. The median and mean days to appear are similar at each temperature indicating that each overall distribution is approximately normal (Zar 1984). After 54 d of incubation at 0.4 and 3.8 °C, only 1 and 3 larvae had appeared on these prunings, respectively (Fig. 1). On day 55 these prunings were transferred to 18 °C and after transfer, 50% of 65 and 33 larvae, respectively, appeared within 8.7 and 6.2 d. These respective median times were 0.2 and 2.7 d shorter than times taken for 50% of the larvae incubated at constant 18.0 °C to appear (Fig. 1). Obviously, there was little or no development occurring at 0.4 °C, while a 2.7-d shorter median emergence time indicates some development probably occurred during 54 d of incubation at 3.8 °C.

Linear Regression-based Emergence Model. Linear regression (Fig. 2) of the relationship between emergence rates (y) and temperatures (x) between 8.8 and 20.9 °C ($y = 0.0066\ x - 0.0067$) provided a reasonable description ($r^2 = 0.57$, $P < 0.05$) of

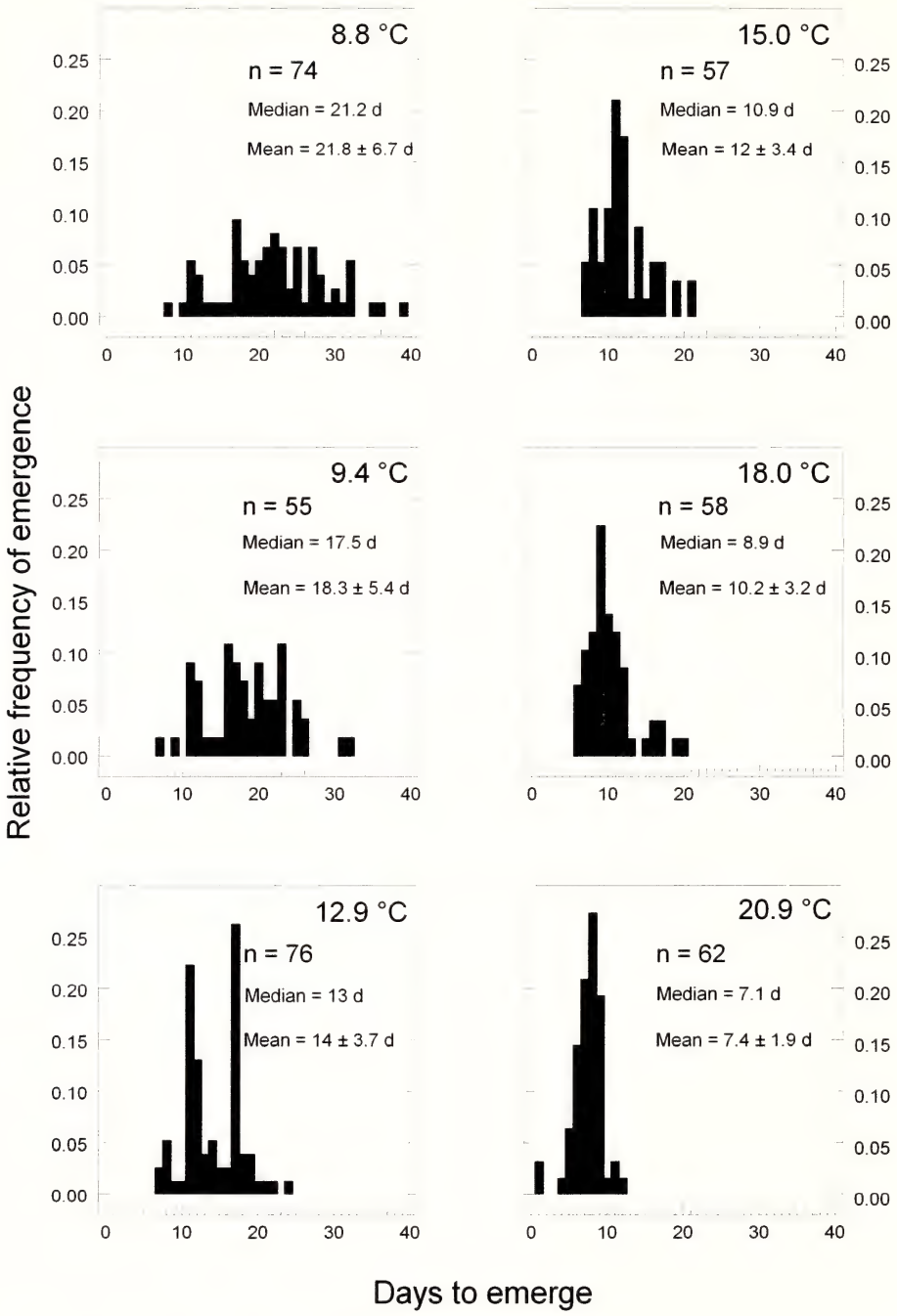

Figure 1. Relative frequency histograms for daily emergence of post-diapause overwintered larvae of *S. ocellana* held at various constant temperatures.

the observed emergence data.

Extrapolation of this line to the *x*-axis provides a theoretical estimate (1.0 ± 0.6 °C) for the lower developmental threshold (T_b). The reciprocal of the slope of this regression line indicates that half the population of ESBM larvae should appear in 154.4 ± 6.7 $DD_{1\,°C}$.

Phenology of Larval Emergence in the Field. Fig. 3 shows relative frequency

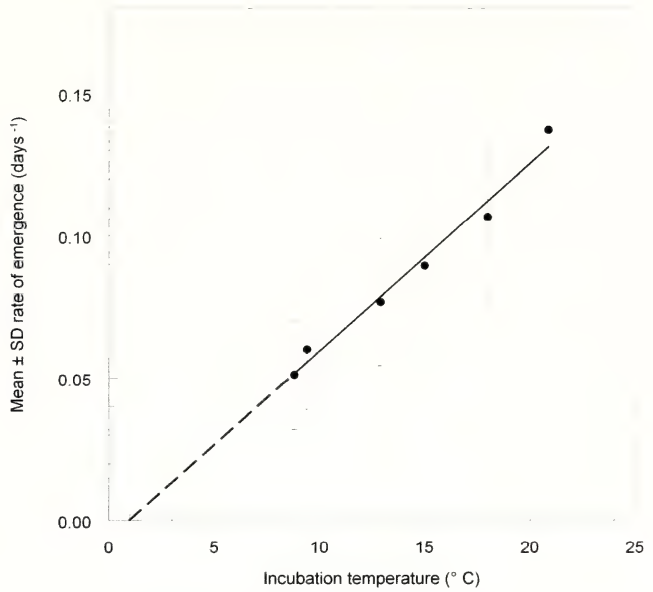

Figure 2. Mean (± SD) emergence rates (solid circles) of post-diapause overwintered larvae of *S. ocellana* at temperatures between 8.8 and 20.9 °C. A least-squares regression line (solid line) was fitted to individual larval emergence rates and extrapolated (dotted line) to the *x*-axis to estimate the base temperature threshold, T_b (1.0 ± 0.6 °C).

curves for daily larval emergence each year. The dates for first emergence ranged from JD 75 (15 March) in 1992 to JD 96 (5 April) in 1996. Fifty and 100% emergence occurred between 0 - 14 and 25 - 35 d, respectively, after the first larva was detected each year. The first ESBM larva was found in the field when McIntosh apple trees were at early green-tip stage of bud development. Fifty and 95% larval emergence consistently occurred in early, tight-cluster and late, pink-bud stages, respectively (Table 2). All larvae had emerged shortly after bloom began. Previous observations on appearance of overwintered ESBM larvae in spring have noted that larvae begin to emerge soon after buds begin to open (Gilliatt 1932, Madsen and Borden 1949, Oatman *et al.* 1962).

Instars of Overwintered Larvae. Among the 66 larvae which appeared on prunings incubated in the laboratory after field collection 1 March 1992 (Table 1), 7, 70 and 23% were fourth, fifth, and sixth instars, respectively. Among the 178 larvae that appeared on prunings during field observation in 1994, 5, 70 and 25% were fourth, fifth, and sixth instars, respectively. The distribution of instars in 1994 was not significantly different ($\chi^2 = 0.614$, df = 2, $P = 0.736$) than the distribution of larval instars observed in the laboratory in 1992. In 1996, 6, 43, and 51% of the 64 larvae observed on prunings in the field were fourth, fifth, and sixth instars, respectively. While the proportion of fourth instars in 1996 was similar to that in 1992 and 1994, the overall distribution of instars in 1996 was significantly different from 1992 ($\chi^2 = 11.778$, df = 2, $P < 0.003$) and 1994 ($\chi^2 = 16.677$, df = 2, $P < 0.001$) because of a greater percentage of sixth instars in 1996. In 1994, 50% of fourth, fifth, and sixth instars appeared in the field by JD 97, 101, and 102, respectively; in 1996 they occurred on JD 106, 108, and 102, respectively. When all larval instars are considered together 50% appeared by JD 100 in 1994, and JD 106 in 1996 (Table 2, Fig. 3).

ESBM has seven larval instars, and on apple, larvae reportedly overwinter in the fifth instar (Gilliatt 1932, LeRoux and Reimer 1959, MacLellan 1978). However, Gilliatt (1932) observed that some larvae also overwinter in the fourth and sixth in-

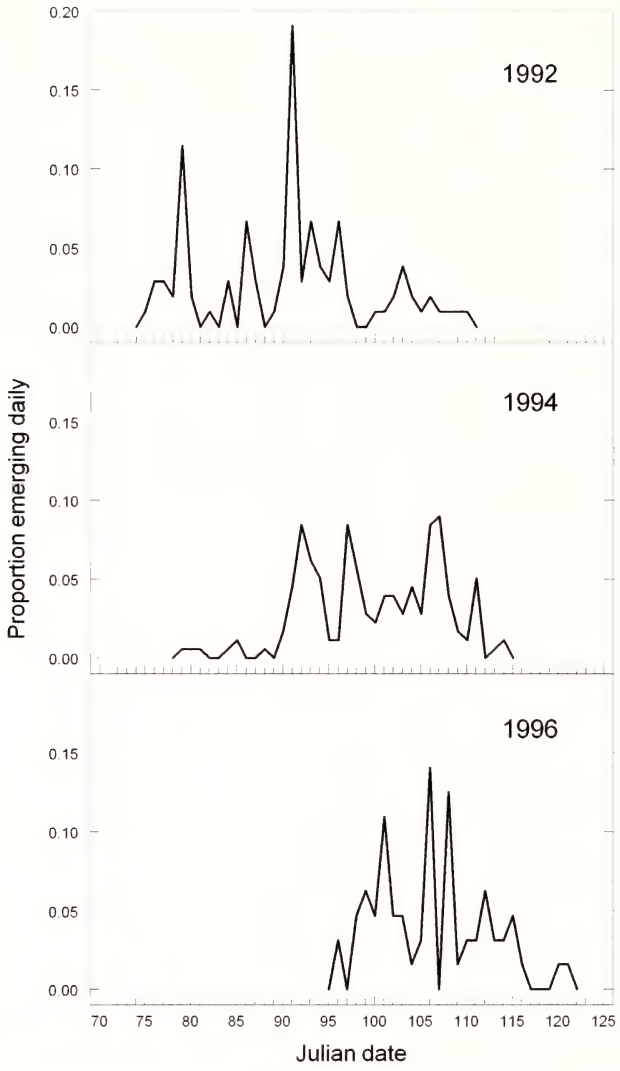

Figure 3. Percent of overwintered larvae of *S. ocellana* emerging daily in 1992, 1994, and 1996. Total larvae emerged in 1992, 1994, and 1996 were 105, 178, and 64, respectively.

stars. Oatman *et al.* (1962) reported that on sour cherry ESBM larvae entered hibernaculae in autumn as third instars and then moulted sometime before appearing in spring. According to Borden and Madsen (1949), on prune ESBM larvae began to construct hibernaculae as fourth instars but moulted during this process, and overwintered as fifth instars. While the present study supports the findings of Gilliatt (1932), because no measures of variation for the mean head capsule widths used to categorize larvae have been provided by any study, it is currently impossible to attach complete certainty to any of these results.

Validating the Linear Regression-based Emergence Model. Using our linear regression model (Fig. 2), a 1 ºC base temperature, and 1 March to start DD accumulations, 50% of ESBM larvae were predicted to appear by JD 87, 90 and 100 in 1992, 1994, and 1996, respectively. Predicted dates for 50% emergence were 4, 10, and 6 d early, respectively (Table 2). The mean (± SD) error between predicted and observed dates of 50% emergence was -6.7 ± 3.1 d. The number of $DD_{1\,°C}$ accu-

Table 2.
Relationship between specific observed cumulative percentiles of emergence of overwintered *S. ocellana* larvae, fruit-bud phenology of McIntosh apple trees and degree-day accumulations above 1 °C ($DD_{1°C}$) starting 1 March in 1992, 1994, and 1996.

Cumulative emergence percentile	Year	Julian date	$DD_{1°C}$	Percentage of fruit buds at each development stage [1]			
				Green tip	Tight cluster	Pink	Blossom
	1992	77	93.2	100			
5%	1994	90	151.8	100			
	1996	98	140.7	100			
	1992	91	177.0	90	10		
50%	1994	100	226.8	90	10		
	1996	106	209.5	95	5		
	1992	106	287.7			100	
95%	1994	111	339.0			100	
	1996	115	276.3		10	90	
	1992	110	329.4			90	10
100%	1994	114	368.3			95	5
	1996	121	320.1			70	30

[1] Percentage based on 20 fruit buds. Fruit-bud phenology based on B.C. Ministry of Agriculture, Fisheries, and Food Tree Fruit Production Guide (1996)

mulating after 1 March to various percentiles of observed emergence of larvae in the field were calculated and shown to be equally variable to 50% emergence (Table 2). Clearly our laboratory-derived linear development model predicted median larval emergence about 20% too early. This deviance may arise because development of ESBM larvae in spring may not be solely related to temperature in the same way life stages like eggs or pupae appear to be (McBrien and Judd 1998). Alternatively, overwintering larvae may have temperature thresholds for movement or feeding activity higher than the T_b we calculated from regression analysis (Fig. 2). If this were the case, overwintering larvae may have completed diapause or post-diapause development by the dates predicted by the regression model, but if temperatures were too low for them to move or feed they may not have appeared on prunings until temperatures were suitable. Accumulating DD before 1 March, when diapause was likely over (Table 1), would only have made the errors of prediction greater, thus it seems a base temperature of 1 °C may be incorrect for predicting larval activity in spring.

Observed DD Accumulations. The CV associated with each observed DD summation calculated using all possible combinations of three starting dates (1 January, February or March) and T_b (1 - 8 °C) to describe observed 50% emergence in the field is shown in Fig. 4. The lowest CV for this one event was obtained with a starting date of 1 January and T_b of 6 °C (Fig. 4). This T_b is not unlike that of two sympatric species of leafroller in the Pacific Northwest, *Pandemis pyrusana* Kearfoot and *Choristoneura rosaceana* Walker, that have a T_b of ≈5 °C (Brunner 1991) and emerge in spring after overwintering as second- or third-instar larvae. The empirically-derived CV-based approach gave a mean of 82.7 ± 3.5 $DD_{6°C}$ from 1 January to 50% emergence, with a CV of 4.3 (Fig. 4), whereas the mean number of $DD_{1°C}$ from 1 March to 50% emergence was

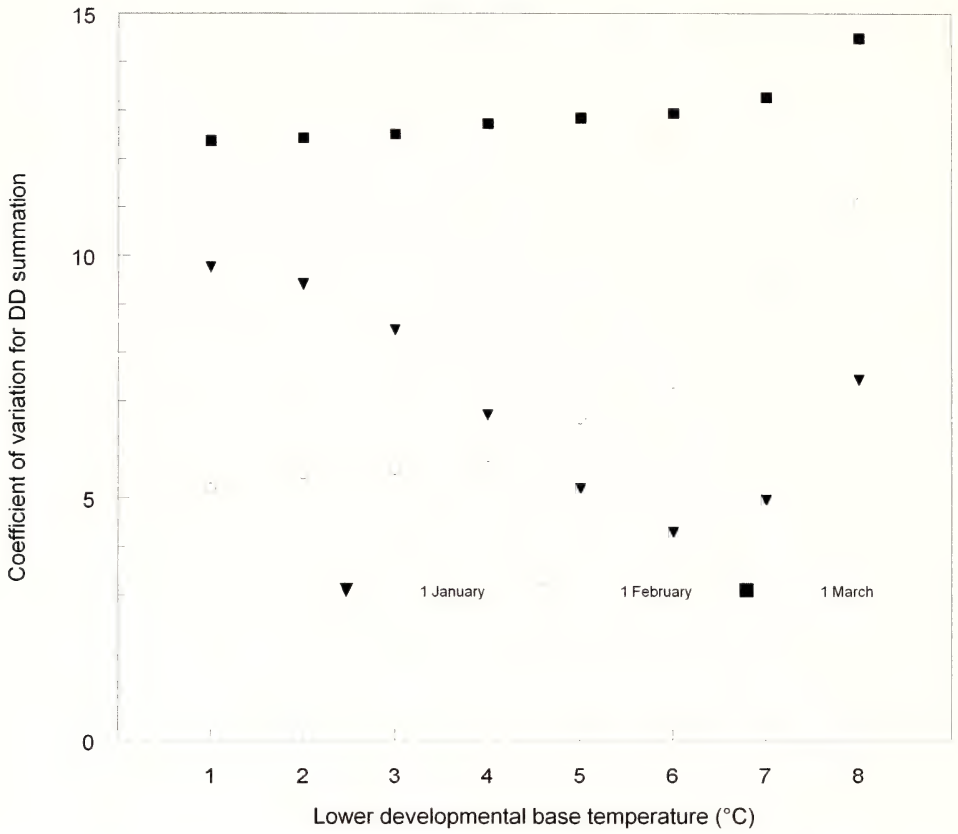

Figure 4. Coefficients of variation associated with three-year average (1992, 1994, and 1996) degree-day summations from various starting dates (1 January, 1 February, and 1 March) to 50% observed emergence of overwintered larvae of *S. ocellana* using different lower base temperatures between 1 and 8 °C.

204.4 ± 25.3 and more variable with a CV of 12.4 (Fig. 4). To provide a methodology for calculating $DD_{6\,°C}$ indices associated with any percentile of observed emergence in the field we plotted observed cumulative larval emergence against $DD_{6\,°C}$ accumulations after 1 January and a cumulative Weibull function accurately described ($R^2 = 0.89$, $P < 0.05$) this relationship (Fig. 5). It should be noted that, although this Weibull function described observed emergence with less variation than the laboratory-derived linear regression and 1 °C T_b (Table 2), the accuracy with which this empirically-derived nonlinear equation can predict future events needs to be validated with independent data. The difference between a T_b derived using this empirical approach and the laboratory-derived DD model may be explained partly by the difference in handling of the prunings used to monitor larval emergence in the laboratory at constant temperatures and to monitor emergence in the field. In the laboratory, prunings were stored at 0.4 °C in total darkness for ca. 3 mo while prunings used to monitor emergence in the field were removed from trees ca. 10 d before larval emergence began naturally. Factors besides thermal summations, such as light, chill units, or moisture which trigger flower bud development, may also determine when overwintering ESBM larvae emerge. Any potentially unknown factors that affect emergence are inherently incorporated within any empirical model even if they are not understood. For whatever reason, our data suggest a developmental model generated from laboratory data only, may not be suitable for

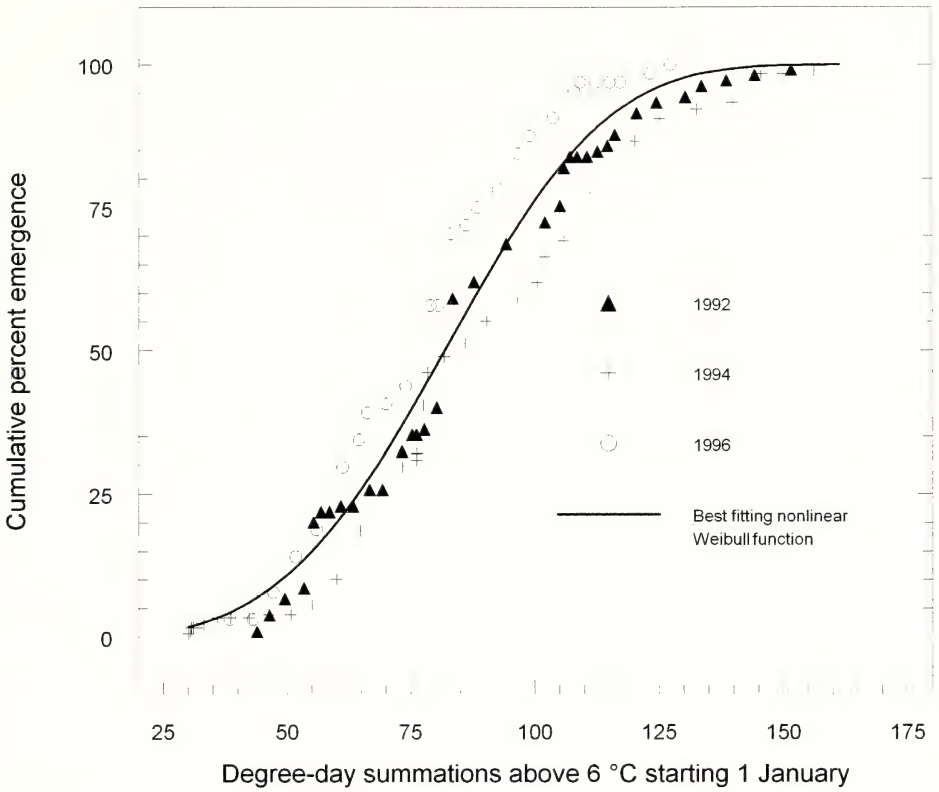

Figure 5. Observed cumulative percent emergence of overwintered larvae of *S. ocellana* in 1992, 1994, and 1996 (data points). Solid line is best fitting ($P < 0.05$) cumulative Weibull function ($\hat{y} = 100 \times (1 - e^{-(x/90.5)})^{3.6}$ used to describe emergence where $x = DD_{6\,°C}$ after 1 January.

describing processes which trigger activity of ESBM larvae during winter and their appearance in spring. On the other hand DD indices generated from observed field data may prove useful in management of ESBM (Fig. 5).

Phenology Models and Management of ESBM. Median emergence of ESBM larvae appeared to be closely linked to fruit-bud phenology of McIntosh apple trees (Table 2), occurring consistently at the early, tight-cluster stage of fruit-bud development. Currently, conventional insecticide applications at tight cluster, pink, or petal fall, or applications of *Bacillus thuringiensis* (Berliner) at full bloom which target leafrollers, are recommended as indirect controls for ESBM in spring (British Columbia Ministry of Agriculture, Fisheries, and Food, 2004). The latter control is one most commonly used by organic producers in the Okanagan and Similkameen Valleys (Judd unpublished data). During this study, emergence of ESBM larvae was complete by early bloom, which is very likely too late for control because most larvae enclose themselves within feeding shelters made from blossoms. Our observations indicate application of residual insecticides to McIntosh apples at the pink stage would have reached 90 - 100% of the larvae (Table 2), well before they enclose themselves in feeding shelters. This is consistent with observations by Madsen and Downing (1968) that azinphosmethyl applied to McIntosh apples in the early pink stage provided good control of ESBM in spring. However, recommendations to spray at pink of McIntosh would not necessarily be appropriate on apple varieties that flower at different dates, which is a common oc-

currence in montane growing regions.

A temperature-based model that can consistently predict 90 - 100% emergence of overwintering ESBM larvae, independent of flowering dates, should have greater application to different apple varieties, years, regions and to host plants other than apple. While this study has not yet provided that model, if the equation given in Fig. 5 can be validated with independent data then it may be useful in predicting any percentile of larval emergence in spring that is deemed necessary for control purposes. Such an equation and models of adult emergence, flight, and oviposition during summer combined with a pheromone-based monitoring program (McBrien and Judd 1998), may provide a more specific management programme for ESBM.

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Seasonal variation in recapture of mass-reared sterile codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae): implications for control by sterile insect technique in British Columbia

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ABSTRACT

In 1992, the Okanagan-Kootenay Sterile Insect Release (SIR) Programme was initiated to eradicate codling moth, *Cydia pomonella* (L.), from montane, fruit-growing valleys in British Columbia (BC), Canada. Excessive damage in 1994, and failures to maintain sterile:wild (S:W) over- flooding moth ratios at 40:1, a target deemed necessary for eradication, led to concern about activity of sterile moths and recommendations to supplement control in spring. Using pheromone-baited wing traps and passive sticky pane traps we monitored operational S:W ratios to determine if they continued to fall below 40:1 post-1994. Seasonal flight activity and recapture of sterile moths was compared with that of wild moths from 7 May - 1 September, 1995 - 1999, in nine commercial orchards in Cawston, BC. Mean weekly catches of wild males in pheromone traps, reflected first- (May) and second-generation (August) peaks of flight activity in orchards supplemented with pheromone disruption, but only a single period of activity in insecticide-supplemented orchards. Weekly catches of sterile moths in these same orchards were always at their lowest in spring, and activity was correlated with seasonal air temperatures. Yearly average S:W ratios in the insecticide-treated orchards ranged from 24:1 - 203:1 in 1995 - 1997. Examining S:W ratios using data from those weeks when wild moths were actually caught, indicates ratios were frequently (29 - 91%) less than 40:1 in spring but S:W ratios fell below 40:1 less often during summer than spring. Passive pane traps also revealed patterns of fewer sterile moth catches, and lower S:W ratios in spring, compared with summer. Our data suggest low overflooding ratios contributed to slower than predicted population reductions, and increased release of sterile moths, of improved quality, between 1995 and 1997 did not significantly increase mean weekly catches or S:W ratios in individual orchards in spring. Therefore, continued application of supplemental insecticides, or a pheromone disruption treatment that reduced catch of moths, but did not significantly affect S:W ratios in spring, is recommended. We conclude that similar analysis of trap data for the entire SIR Programme (1994 - 2004) and correlations with damage would provide recommendations for the best use of sterile insects as part of any future area-wide codling moth management programme.

Key Words: Codling moth, sterile insect technique, flight activity, sterile:wild ratios

INTRODUCTION

In 1992, the British Columbia Fruit Growers' Association and Regional District governments launched a multimillion dollar Sterile Insect Release (SIR) Programme in the Creston, Okanagan and Similkameen Valleys of British Columbia

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(BC), Canada with an objective to eradicate codling moth, *Cydia pomonella* (L.), from these montane, fruit-growing valleys by 2000 (Dyck and Gardiner 1992). This area-wide programme was the culmination of 30 years of basic research, small implementation trials and much planning (Proverbs 1971, 1982; Proverbs *et al.* 1966, 1977, 1982; Dyck *et al.* 1993).

Originally the programme was divided into three distinct phases: 1) *Pre-release Sanitation*, 2) *Sterile Moth Release*, and 3) *Surveillance Monitoring and Protection.* During Phase 1 growers were required to reduce wild codling moth populations to levels resulting in 0.1 - 0.3 % damage at harvest, considered equivalent to about 100 overwintering larvae per ha (Dyck *et al.* 1993). Operationally, it was anticipated these measures would equate to adult populations of no more than two wild moths per pheromone trap per week during periods of peak emergence (Bloem and Bloem 2000). In Phase 2, the programme would make weekly releases of sterile moths in all commercial orchards in order to maintain sterile:wild (S:W) overflooding moth ratios at 40:1, a target that extensive research demonstrated was necessary to eradicate populations in three years (Proverbs 1971, 1982; Proverbs *et al.* 1982). In Phase 3, an area-wide pheromone trapping programme would be implemented post-2000 to prevent reinfestation of pest-free areas through early detection and targeted controls. These three phases were to be implemented sequentially in each of two zones. Zone 1, which included the Okanagan from Osoyoos to Summerland, Similkameen and Creston Valleys, entered Phase 1 in 1992 and was to recieve sterile moths under Phase 2 from 1994 - 1996. Zone 2 from Peachland to Salmon Arm inclusive, was scheduled to enter Phase 1 in 1995 and receive moths from 1997 - 1999 (Dyck *et al.* 1993).

Following a two-year pre-release sanitation programme in Zone 1, the first sterile moths were released in May 1994. Almost immediately it was noted that weekly S:W ratios from pheromone trap catches in individual orchards were less than the 40:1 target. Unfortunately, in previous sterile insect implementation trials in BC (Proverbs 1982; Proverbs *et al.* 1966, 1977, 1982;), codling moth S:W ratios were almost always presented as averages for regions, generations or seasons. These coarse-grained values made it difficult to know what ratios should be expected on a weekly basis in individual orchards and whether there should be concern for the long-term control effort. In this regard, similar implementation trials in Washington state (White *et al.* 1976) showed that S:W ratios within a single 130-ha orchard ranged from 54:1 to 938:1 when all traps were averaged over the season, but when examined individually, 21 of 38 total traps showed at least one weekly ratio that was less than 20:1. Furthermore, 78% of all codling moth damage was found within 152 m of these 21 traps. Therefore, to achieve area-wide population reduction and suppression of codling moth in a fruit-growing region like BC, where there are numerous, small, noncontiguous orchards, it is important to maintain appropriate S:W ratios in individual orchards and traps, especially during periods of peak emergence of wild moths. Any critical assessment of the annual progress or success of a sterile insect programme in BC should be based on an analysis that reveals the extent to which appropriate S:W ratios are being achieved in individual sites and presented as a proportion of all orchards being treated.

Our original objectives in this study were to document operational S:W ratios in several orchards in an effort to determine whether they were falling below the 40:1 target after 1994, and if so, to determine the extent to which inadequate ratios were resulting from large wild populations, thus justifying need for supplemental controls, or were resulting from poor recapture of sterile moths. A further rationale in publishing these data now, is that these data and analyses of this type are needed to make objective decisions about the design of a sustainable area-wide codling moth

control programme post-2005 (Dendy *et al.* 2001). Our analyses may be useful in making decisions on the best uses of sterile codling moths in BC and other parts of the world where application of this technique is currently being considered.

MATERIALS AND METHODS

Test Sites and Sterile Insect Delivery. Studies were carried out in four conventionally- and five organically-managed apple orchards in Cawston, BC, located in the Similkameen Valley, within Zone 1 of the Okanagan-Kootenay SIR Programme (Dyck *et al.* 1993). Orchards ranged in size from 1 - 3 ha and were composed of mixed 'McIntosh', 'Spartan', 'Delicious' and 'Golden Delicious' apple varieties planted at densities of 600 - 900 trees / ha with tree × row spacings of 2.4 - 4.6 × 4.6 - 5.5 m, respectively. Orchards were chosen because of their known history (Judd *et al.* 1996, 1997) and because they were part of the original sterile insect implementation trials conducted by Proverbs *et al.* (1982).

While our trials were being conducted, conventional growers were asked to apply insecticidal controls during flight of first-generation adult codling moths in May and June, which most growers did in 1995 - 1997 (Bloem and Bloem 2000). Organic growers did not apply insecticides, but they supplemented release of sterile males by applying Isomate-C and Isomate CM/LR (Pacific Biocontrol Corp., Vancouver, Washington, USA) in 1995 - 1996 and 1997 - 1999, respectively, at a rate of 500 - 1000 dispensers / ha, as pheromone treatments to disrupt mating of wild codling moths (Judd *et al.* 1996, 1997; Judd and Gardiner 2004). Pheromone treatment was required because organic growers did not want to lose certification by applying conventional insecticides. This application presented an opportunity to determine what impact a pheromone-based mating-disruption treatment might have on flight activity of sterile moths and S:W ratios.

The original plan was to deliver 1000 mixed-sex sterile codling moths in each ha of orchard two times each week (Dyck *et al.* 1993). However, Bloem and Bloem (2000) stated that on average orchards were receiving 2250 moths / ha / week in 1994, and as many as 3750 moths / ha / week in 1997, although it is not clear whether these numbers refer to mixed-sex moths, or males only. Exactly how many sterile moths reached our study orchards is unknown, but all orchards were visited twice weekly by the same release drivers throughout each year and therefore, should have received similar numbers of moths. Beginning in late April or early May and continuing until mid-September, moths were distributed in every fifth or ninth row of orchard approximately 25 - 30 m apart. Chilled moths irradiated as described by Bloem and Bloem (2000) were dispensed by gently blowing them onto the ground beneath trees from a small hopper and fan unit (McMechan and Proverbs 1972) mounted on the front end of an all-terrain vehicle (Bloem and Bloem 2000).

Monitoring Codling Moth Seasonal Flight Activity. From 1995 through 1999, seasonal flight activity and capture of sterile and wild male codling moths was assessed using pheromone-baited traps. Two to six Pherocon 1-CP style, sticky wing traps (Phero Tech Inc., Delta, BC) baited with the codling moth sex pheromone codlemone, (*E*,*E*)-8,10-dodecadien-1-ol (99% isomerically pure, Shin-etsu, Fine Chemicals Division, Tokyo, Japan) were used in each orchard. A minimum of two traps and a maximum of two traps per ha were used in each orchard. Traps were hung ca. 1.5 - 2.0 m above ground near the centre of each orchard on 7 or 8 May (Julian Day 128) and were checked weekly until 1 September. Trap positions remained fixed within orchards across seasons. Trap bottoms were replaced weekly and pheromone baits were changed every third week. Sterile codling moths were identified by an internal red dye sequestered from the artificial diet on which they

were reared (Brinton *et al.* 1969).

All pheromone lures were prepared by dispensing a 200 µl solution of codlemone dissolved in dichloromethane solvent into wells of red rubber septa (Aldrich Chemical Company Inc., Milwaukee, Wisconsin, USA). After loading, septa were air dried for 18 h at 23 °C in a fume hood and stored at 0 °C until pinned to the inner side of trap lids in the field. Septa used in conventional orchards were loaded with 1 mg of codlemone and septa used in pheromone-treated orchards were loaded with 10 mg of codlemone because the former are less attractive in Isomate-treated orchards (Judd *et al.* 1996).

In 1996, catches of sterile and wild, male and female codling moths were also monitored using passive pane traps (Weissling and Knight 1994) to measure relative activity of sterile and wild moths independent of response to pheromone lures. Pane traps consisted of vertically-oriented, semirigid, clear acetate plastic squares (30 × 30 cm) that were coated with STP oil treatment (First Brands Corporation, Scarborough, Ontario, Canada) to capture alighting insects. Sticky panes were clamped 3 m above ground to an upright iron rod abutting tree trunks. Eight to 30 pane traps were deployed evenly in a grid pattern throughout each orchard. Due to their cumbersome nature and need for frequent maintenance, pane traps were only used in 1996 and were placed in the field every other week, and only for a three-day sampling interval, Tuesday through Thursday.

Orchard Temperatures. Hourly air temperatures throughout this study were recorded at a centrally-located orchard in Cawston, BC from 1 May through 1 September each year. Temperature readings were made using a two-channel DP-212 Datapod (OmniData™, Logan, Utah, USA) housed in a 1 m high Stevenson screen. Daily degree-day (DD) summations above a 10 °C developmental base temperature and below a 31 °C upper developmental threshold were calculated by fitting a sine wave (Allen 1976; case 4) to daily air temperature minima and maxima using the computer program described by Higley *et al.* (1986).

Data Analyses. Codling moth catches in all traps within an orchard were summed each week. Median catches per trap per orchard in each generation and year were compared using a Kruskal Wallis non-parametric analysis of variance followed by Dunn's multiple comparisons test for ranked data (Zar 1984). Some paired frequency data were analyzed using Fisher's exact test and χ^2 tests where appropriate. Regression analyses was used to relate weekly and seasonal catches to temperature. All statistical tests were performed using SigmaStat® (Version 3.0.1, SYSTAT Software Inc., Richmond, California, USA) and an experimental error rate of $\alpha = 0.05$.

RESULTS

Flight Activity of Wild Moths. Mean weekly catches of wild codling moths in conventional insecticide-treated orchards in 1995 - 1999 peaked at 12, 4.5, 0.75, 0.4 and 0.2 moths / trap / week during the first four weeks of each trapping season, respectively (Fig. 1A). Catches of wild moths in insecticide-treated orchards were always lower in the second half of the season (Fig. 1A) and near zero after 1996. Catches of early-season, first-generation wild moths however continued into 1999 (Fig. 1A). After 1996, catches of wild moths in insecticide-treated orchards never went above the anticipated maximum threshold of 2 wild moths / trap / week, and during the five-year monitoring period they were less than this threshold 97.5% of the time.

In contrast to insecticide-treated orchards, trap-catch curves in pheromone-treated orchards reflected both first- and

second-generation peaks of wild moth flight activity in 1995 and 1996 (Fig. 1B). Total catches in the second half of 1995 were even greater than the first half. However, after 1996, catches of wild codling moths were extremely low under pheromone-treatment and no wild moths were caught in any pheromone-treated orchard in 1999 (Fig. 1B).

Flight Activity of Sterile Moths. A plot of mean ± standard error (SE) weekly catches of sterile moths from 1995 - 1999 in conventional insecticide-treated orchards reveals a pattern of low catches during the early season and increasing catches later in the season (Fig. 2). Mean catches of sterile moths in the first two weeks of the season (Fig. 2) were often below the 40 moths / trap / week needed to ensure a 40:1 S:W ratio if only a single wild moth were caught. On average, it was not until week 8 that catches were consistently greater than 80 moths / trap / week needed to ensure a 40:1 S:W ratio if the programme threshold of 2 wild moths / week were reached. There was a significant linear ($r^2 = 0.87$, $P < 0.05$) increase in weekly catches of sterile males in weeks 1 - 8, but catches over the entire season were described more accurately ($R^2 = 0.93$, $P < 0.05$) by a sigmoid curve (Fig. 2). Increasing catches in weeks 1 - 8 were

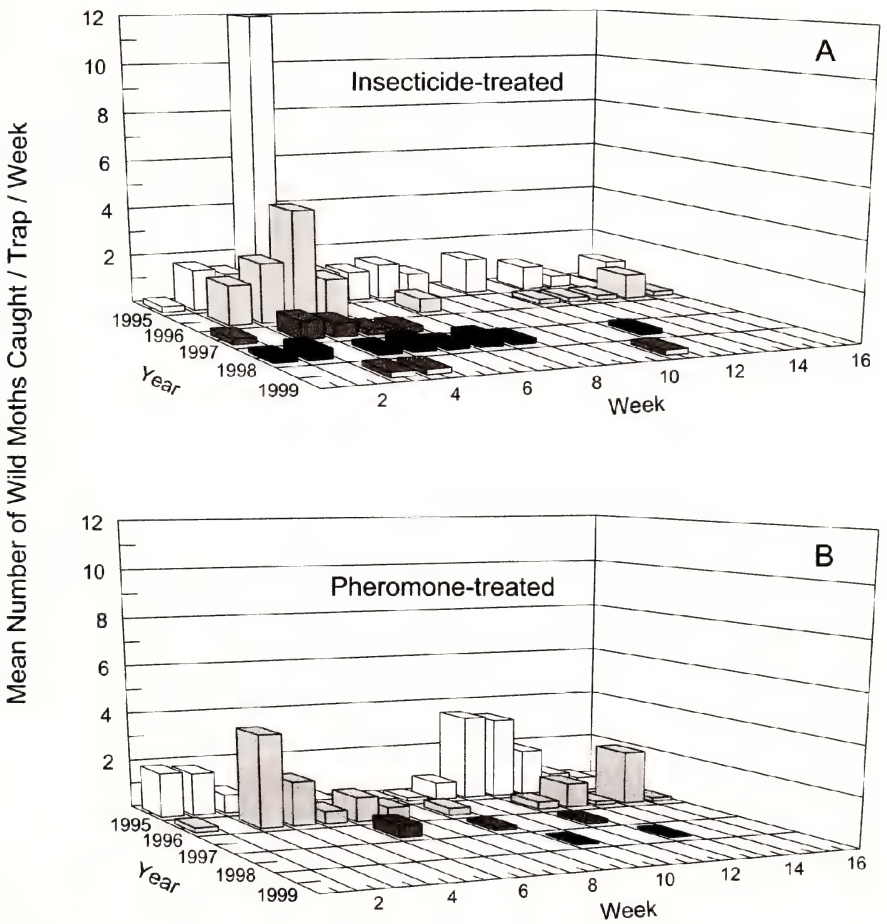

Figure 1. Mean weekly catches of wild male codling moths in pheromone-baited wing traps in insecticide-treated conventional (A) and pheromone-treated organic (B) apple orchards under management of the Okanagan-Kootenay SIR Programme from 1995 - 1999, in Cawston, BC. n = 4 conventional and 5 organic orchards and 2 - 6 traps / orchard.

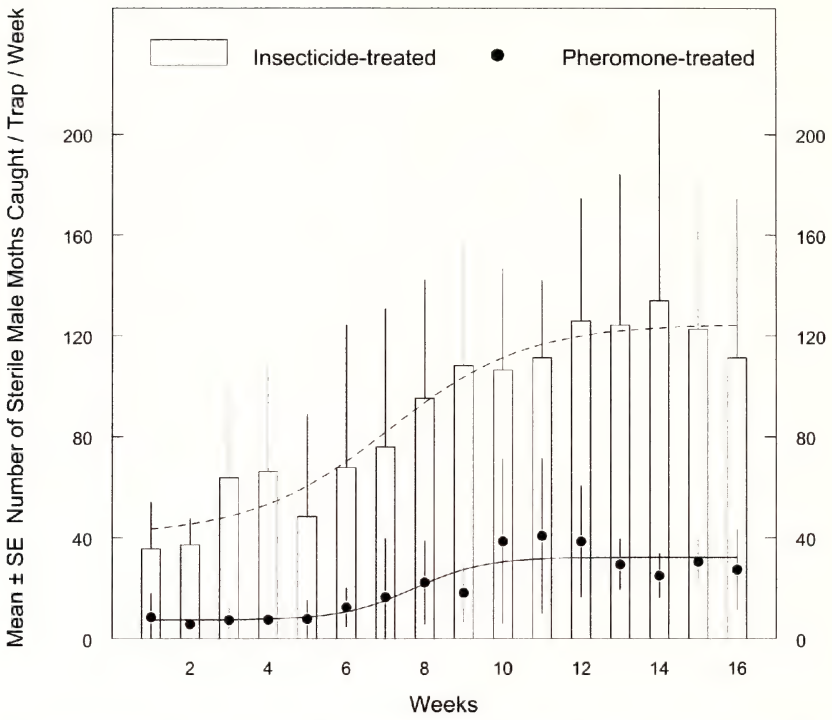

Figure 2. Comparison of mean (± SE) weekly catches of sterile male codling moths in pheromone-baited wing traps in insecticide-treated conventional (bars) and pheromone-treated organic (dots) apple orchards under management of the Okanagan-Kootenay SIR Programme from 1995 - 1999, in Cawston, BC. Curves represent nonlinear regression lines fitted to mean catches in insecticide- ($124.7 / [1 + e^{-(x - 7.1) / 1.75}]$) and pheromone-treated orchards ($32.2 / [1 + e^{-(x - 7.72) / 0.9}]$).

correlated ($r = 0.661$, $P < 0.05$) with concurrent seasonal increases in mean weekly temperatures.

Although weekly catches of sterile moths in pheromone-treated orchards were reduced significantly (ca. 78%) over catches in insecticide-treated orchards, they revealed a similar pattern of low early season catches that increased as the season progressed (Fig. 2). Catches of sterile males regularly reached as many as 40 moths / trap / week after week 9. Again, while there was a significant linear ($r^2 = 0.641$, $P < 0.05$) increase in catches of sterile males in pheromone-treated orchards during weeks 1 - 8, catches over the entire season were better described ($R^2 = 0.83$, $P < 0.05$) by a sigmoid curve (Fig. 2).

The median number of sterile moths caught in conventional orchards varied less among years than between generations within years (Table. 1). There was no significant difference (Dunn's test $\alpha = 0.05$) in the median number of sterile moths caught during weeks 1 - 8 in 1995, 1996, 1997 and 1999 (Table. 1). In 1998 however, significantly more sterile moths were caught, mean temperatures were higher, and more $DD_{10\,°C}$ accumulated during the first eight weeks of the season than any other year (Table 1). In each of the five years, median trap catches in weeks 1 - 8 (first generation) were less than median catches in weeks 9 - 16 (second generation). In each year, the ratios of second-generation to first-generation median trap catches were significantly ($P < 0.05$) different than an expected 1:1 ratio under a null hypothesis of equal probabilities of catch (Table 1), and over five years sterile moths were 3.9-fold more likely to be caught in second than in first generation.

Table 1.
Median number of sterile male codling moths caught per trap per week and degree-day (DD) accumulations during first- and second-generation wild moth flight activity and median yearly catches and sterile:wild (S:W) overflooding moth ratios in four insecticide-treated conventional apple orchards receiving sterile codling moths under management of the Okanagan-Kootenay SIR Programme, in Cawston, BC, 1995 - 1999.

Year	Adult generation[1,2]				Generational trap catch ratios[3]	Median yearly total catch	Yearly S:W overflooding ratios
	First (weeks 1 - 8)		Second (weeks 9 - 16)				
	Catch	$DD_{10°C}$	Catch	$DD_{10°C}$	Second / First		
1995	27.4a	463	53.2 a	584	1.9 *	40.3a	24
1996	42.7a	339	175.1 b	674	4.1 *	108.9b	139
1997	37.9a	427	72.0 a	611	1.9 *	56.4a	203
1998	112.9b	512	204.9 b	757	1.8 *	158.9b	794
1999	65.3a	374	168.5 b	653	2.6 *	116.9b	275
1995-1999	42.7	423	168.1	656	3.9 *	108.9	287

[1] Trap-catch medians for a generation within a column followed by different letters are significantly different (Dunn's test $\alpha = 0.05$) following a significant ($P < 0.05$) Kruskal-Wallis Test.
[2] $DD_{10°C}$ totals above 10 °C accumulated for first generation from Julian Day 128 - 184 and for second generation from Julian Day 185 - 241.
[3] Asterisks indicate trap-catch ratios are significantly different ($P < 0.05$) from 1:1 by a χ^2 test on actual trap catches.

Sterile:Wild Moth Ratios. S:W ratios presented as yearly averages for all orchards suggest overflooding ratios were well above 40:1 in all years except 1995 (Table 1), steadily increasing from 1995 - 1998. However, restricting analysis of S:W ratios in the insecticide-treated orchards to those weeks when wild moths were actually caught (Table 2), indicates ratios during the first eight weeks of the season were less than 40:1, 91% of the time in 1995 (21 of 23 orchard-weeks), 60% in 1996 (9 of 15 orchard-weeks), and 29% in 1997 (2 of 7 orchard-weeks). Closer examination of S:W ratios in these orchards shows that during the first four weeks of the trapping season, when wild catches peaked (Fig. 1A), S:W ratios never reached 40:1 in 1995, did so once in 1996 and only twice in 1997. In one of the three orchards where wild moths were caught in 1998 (Table 2), S:W ratios fell below 40:1 in both weeks this occurred. It was not until 1999, when wild catches were near zero (Fig. 1A), that a 40:1 S:W ratio was achieved in all orchards during the first eight weeks of the season (Table 2). Similar analysis performed in the second half of the season indicates S:W ratios fell below 40:1 about 39% of the time over the five years (9 of 23 orchard weeks), which was significantly less often than during the first eight weeks (Table 2).

S:W ratios in pheromone-treated orchards were only analyzed for 1995 and 1996 because few or no wild moths were caught in these orchards in 1997 - 1999 (Fig. 1B). In spite of yearly reductions (4.5-fold) in catches of sterile moths in pheromone-treated relative to insecticide-treated orchards (Fig. 2), S:W ratios during the first eight weeks of 1995 were similar in both sets of orchards (Fig. 3A-B). This similarity was surprising because in most weeks, wild catches were greater in pheromone- than in insecticide-treated orchards where wild catches went above 2 moths / trap / week only once. While S:W ratios in pheromone-treated orchards never went above 40:1 during flight of first-generation

Table 2.

Frequency of weeks during the first (weeks 1 - 8) and second generation (weeks 9 - 16) of wild codling moths when sterile:wild ratios in pheromone-trap catches were less than 40:1 (numerators) for those weeks when wild moths were caught (denominators) in each of four insecticide-treated conventional orchards receiving sterile codling moths under management of the Okanagan-Kootenay SIR Programme, in Cawston, BC, 1995 - 1999.

Year	Adult generation	Orchards				Yearly frequency totals	Fisher's Exact Test *P*-value[1]
		1	2	3	4		
1995	1st	5 / 5	7 / 8	5 / 5	4 / 5	21 / 23	0.0345
	2nd	1 / 2	3 / 6	1 / 2	3 / 4	8 / 14	
1996	1st	2 / 2	1 / 5	2 / 3	4 / 5	9 / 15	0.0333
	2nd	0 / 1	1 / 4	0 / 0	0 / 4	1 / 9	
1997	1st	0 / 0	2 / 4	0 / 1	0 / 2	2 / 7	ns
	2nd	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	
1998	1st	0 / 0	0 / 3	2 / 2	0 / 2	2 / 7	ns
	2nd	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	
1999	1st	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	ns
	2nd	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	
Orchard totals	1st	7 / 7	10 / 20	9 /11	8 / 14	34 / 52	0.0494
	2nd	1 / 3	4 / 10	1 / 2	3 / 8	9 / 23	

[1]Fisher's Exact Test (2 × 2 contingency table) of the null hypothesis of equal frequencies in each generation during which the sterile:wild ratios are < 40:1 using yearly frequency totals from all orchards. ns = $P > 0.05$

wild moths in 1995 (Fig. 3B), they were only marginally better in the insecticide-treated orchards where ratios reached 40:1 once (Fig. 3A). Note however, this one occurrence was in the week when wild moths were near their lowest, and followed a week when catches of wild moths peaked but catches of sterile moths inexplicably dipped (Fig. 3A). S:W ratios in pheromone-treated orchards fell below 40:1 most of the second half of 1995 (Fig. 3B) because catches of wild moths increased. Late-season increase in wild-moth activity in these orchards may be caused by using more attractive 10 mg lures and waning effects of pheromone disruption dispensers. This apparent increase in activity of second-generation wild moths was a temporary aberration in pheromone-treated orchards. Over five years, total seasonal catches of wild moths declined faster in pheromone-treated orchards than they did in the insecticide-treated orchards (Fig. 1).

Pane Traps *versus* Pheromone Traps. Catches in pane traps from individual orchards were too low to show any significant weekly patterns or conduct any meaningful statistical analyses on catches of either sterile or wild codling moths, so catches were pooled across orchard treatments. S:W ratios on pane traps in weeks 1 - 8 (first generation) are compared to those in weeks 9 - 16 (second generation) and ratios on pane traps are compared with those reflected by pheromone trap catches (Fig. 4). In the insecticide-treated orchards (Fig. 4A) catches in pane traps reflect the same pattern as pheromone traps, low S:W ratios were observed in weeks 1 - 8 and higher S:W ratios in weeks 9 - 16. S:W ratios on pane traps increased 6.2-fold while those in pheromone traps only increased 4.8-fold across generations (Fig. 4A), suggesting activity-driven responses to the former were more important than increased pheromone responses to the latter.

In pheromone-treated orchards (Fig.

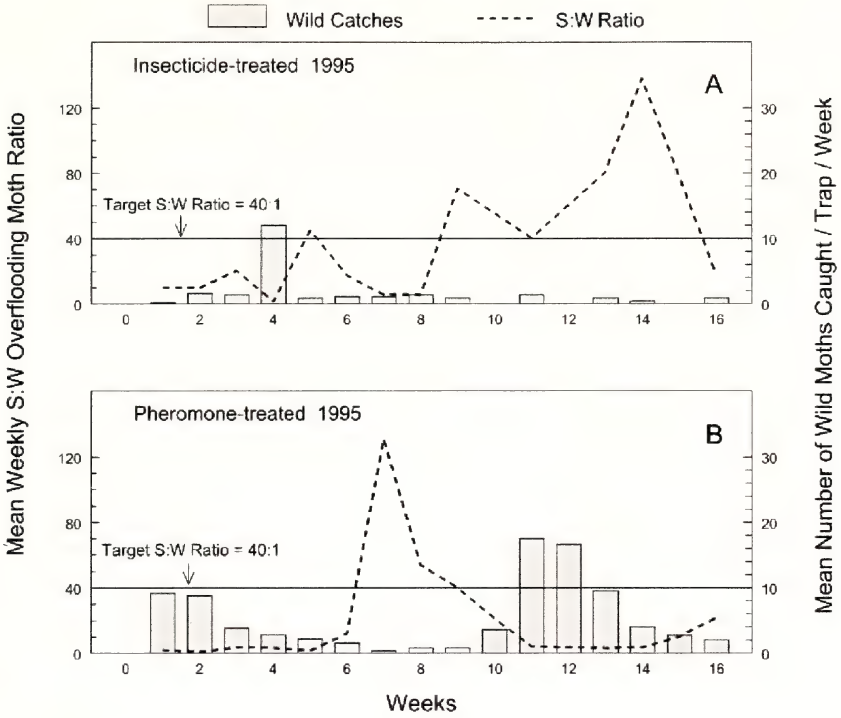

Figure 3. 1995 mean weekly catches of wild male codling moths and sterile:wild moth ratios in pheromone-baited wing traps in insecticide-treated conventional (A) and pheromone-treated (B) organic apple orchards under management of the Okanagan-Kootenay SIR Programme, in Cawston, BC. n = 4 conventional and 5 organic orchards and 2 - 6 traps / orchard.

4B) catches with both pane traps and pheromone traps once again reflect the established pattern of low S:W ratios in weeks 1 - 8 and higher ratios in weeks 9 - 16. However, in pheromone-treated orchards S:W ratios on pane traps showed the same cross-generation increase (2.1-fold) as pheromone traps (2.3-fold), reflecting suppression of sterile male response to pheromone-baited traps in orchards under pheromone-based mating disruption (Fig. 2), which artificially limits ratios.

Catches of females on pane traps were too low to ascribe any specific seasonal pattern in their response.

DISCUSSION

Decades of research examining use of sterile insect technique to control codling moth have identified the need to maintain 40:1 S:W overflooding moth ratios in order to achieve population reduction, and have further emphasized the importance of doing this during the first generation because the reproductive potential of the species is lowest at this time of year. Our detailed examination of seasonal flight activity and recapture of male codling moths in nine commercial orchards under management by sterile insect release showed significant within-season variation over five years, often resulting in S:W ratios less than 40:1. Low S:W ratios were most pronounced in, but not restricted to, the first eight weeks of the trapping season from May through June (Fig. 3; Table 2), the period of wild-moth activity referred to as first generation (Madsen and Procter 1985).

In this study, catches of first-generation wild moths generally peaked during the first four weeks of the season in late May

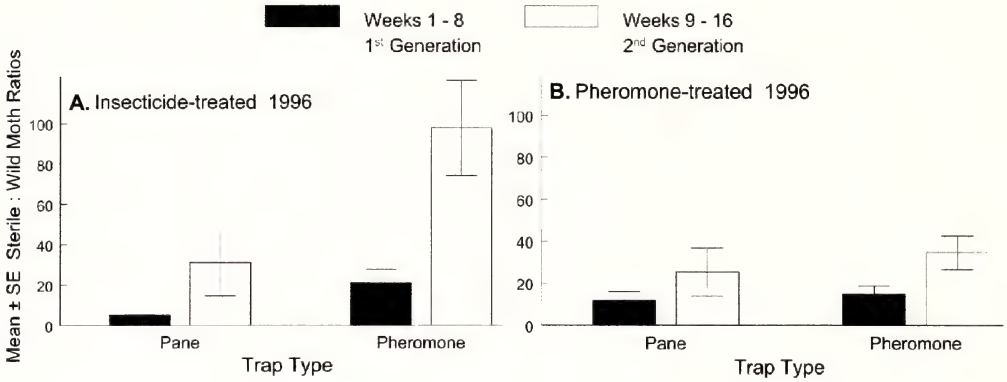

Figure 4. 1996 mean (± SE) sterile:wild moth ratios from catches in pheromone-baited wing traps and sticky pane traps during first- (weeks 1 - 8) and second-generation (weeks 9 - 16) flight-activity periods for wild moths in insecticide-treated conventional (A) and pheromone-treated organic (B) apple orchards under management of the Okanagan-Kootenay SIR Programme, in Cawston, BC. n = 4 conventional and 5 organic orchards and 2 - 6 pheromone traps and 8 - 30 pane traps / orchard.

or early June (Fig. 1A-B) and when it occurred, second-generation catches peaked in weeks 11 - 12 during early August (Fig. 1B). In contrast, catches of sterile moths (Fig. 2) were at their lowest in the first four weeks of the season, increasing thereafter and reaching a plateau around week 10 in July. The observed sigmoid pattern (Fig. 2) in recaptures of sterile moths might be explained by a combination of factors: (1) released moths that live longer than a week may accumulate with subsequent weekly releases, (2) there may be greater mortality of sterile moths in spring perhaps because of more frequent application of insecticides or cooler temperatures, (3) sterile moths may be less responsive to pheromone traps in spring than summer, (4) sterile moths may be less active under cool spring temperatures than they are in summer, or (5) trap catches may be limited in summer because trap bottoms become saturated with moths reducing apparent late season activity. Whatever the cause(s) it seems likely this seasonal pattern is inherent to activity of sterile moths because a similar pattern was observed in both insecticide- and pheromone-treated orchards, where the latter received no pesticides and where traps never became saturated in moths (Fig. 2).

Response to temperature likely explains much of this seasonal variation because the greatest number of sterile moths was caught in 1998 (Table 1), the warmest spring in this five-year study, and a significant linear correlation was found between mean weekly catches and mean temperatures. S:W ratios in catches on passive pane traps were also greater during weeks 9 - 16 compared with weeks 1 - 8 (Fig. 4A), lending support to the idea that sterile moths are simply less active than wild moths in cool spring weather (Bloem and Bloem 1996; Bloem *et al.* 1998). The lack of any significant difference in spring-time S:W ratios in pheromone- and insecticide-supplemented orchards when measured by pheromone traps (compare Fig. 4A and 4B), is further indication of a seasonal difference in activity. Catches of sterile moths are suppressed by pheromone treatment in summer (Fig. 2) and this reduces S:W ratios relative to insecticide-treated orchards when measured using pheromone traps during weeks 9 - 16 (compare Fig. 4A and 4B). In spring the opposite occurs. Activity of sterile moths as evidenced by catches in the insecticide-treated orchards (Fig. 2) is already low in spring. Therefore, full expression of disruption is not seen in spring and S:W ratios are more similar in pheromone- and insecticide-treated orchards when measured using pheromone traps (compare Fig. 4A and 4B).

Inactivity of sterile moths may be an artifact of laboratory-rearing conditions. Proverbs (1971) stated, although no data were presented, that moths reared at high constant temperatures, as they are in the current SIR Programme (Bloem and Bloem 2000), are less active in spring than wild moths, and males reared at constant temperatures are apparently less responsive to synthetic pheromone than males reared under fluctuating temperatures, especially in spring (Proverbs 1982). Hutt (1979) confirmed that recapture of sterile codling moths in pheromone traps increased after insects were reared at fluctuating temperatures. However, Bloem *et al.* (1998) did not see an affect of fluctuating rearing conditions on pane-trap catches in spring and they saw greater catches in pheromone traps in fall than in summer, even though dusk temperatures were cooler in fall. These apparent contradictions suggest additional factors may be at work. Whether temperature impacts flight activity of sterile moths directly, or indirectly through rearing, or somehow modulates their response to pheromone sources remains to be determined. It should be noted that mass-rearing of sterile moths under fluctuating temperature regimes was tested but deemed impractical in the current Osoyoos rearing facility (Bloem *et al.* 1998).

When the SIR Programme was designed and production capabilities of the rearing facility were considered (Dyck *et al.* 1993), it was calculated that production and release should be able to ensure 40:1 overflooding ratios if the *Pre-release Sanitation* phase could reduce populations of wild codling moth to levels resulting in no more than 0.1- 0.3% harvest damage. It was assumed this level of damage would result in no more than 2 wild moths / trap / week / orchard and initial release numbers were generated around this threshold (Bloem and Bloem 2000). Assuming catches in pheromone traps reflect operational ratios of sterile and wild moth populations, traps need to show catches of 80 sterile males / trap / week, if the SIR Programme is going to maintain 40:1 ratios when wild populations are this large. Our data show that during the first eight weeks of the season this target was only achieved in 1998. These data strongly suggest populations of wild moths should be reduced to levels resulting in no more than 1 wild moth / trap / week at peak flight to ensure S:W ratios are consistently at or above the 40:1 target during spring.

Alternatively, if wild populations are not reduced, 40:1 S:W ratios might be maintained by releasing more sterile moths when wild populations are reaching 2 moths / trap / week. Intuitively, this sounds like a reasonable approach but our analysis suggests it may be questionable in spring. For example, recaptures of sterile moths released in 1995 and 1997 were not significantly different in either generation (Table 1). This is surprising because the SIR Programme reportedly released 1.7 times more sterile moths in 1997 than 1995, and fewer insecticides were applied against codling moth in 1997 than in 1995. With catches of sterile moths averaging 37.9 / trap / week during first generation in 1997, the best that might have been achieved was a 19:1 S:W ratio if 2 wilds / trap / week were caught. At this level of wilds even median catches in summer 1997 (Table 1) would not have produced an overflooding ratio of 40:1. These observations strongly suggest that increasing production and release of sterile moths in 1997 had no demonstrable impact on recapture of sterile moths in our study orchards, particularly in spring, and these increases would rarely have resulted in adequate S:W ratios in individual orchards unless wild populations were reduced well below 2 moths / trap / week.

As a caveat, it should be noted that more sterile moths were produced and released in 1997 than in 1995, but there are no records to verify the number of moths release drivers delivered to any particular orchard. It can also be argued that operational S:W ratios might have appeared larger in this study had we used a trap which was less susceptible to saturation with sterile moths than small wing traps.

However, while trap saturation may play a role in the plateau seen on catch curves in summer (Fig. 2), which conceivably places upper limits on S:W ratios, trap saturation was never an issue in spring, nor on pane traps, nor in pheromone-treated orchards showing the same trends. One of the reasons we used wing traps was because these traps were the standard for the operational programme being assessed.

In spite of suboptimal S:W ratios, mean seasonal catches of wild moths declined from 1995 - 1999 in orchards receiving sterile moths and supplemental insecticides in spring. After three years, second-generation moths were almost undetectable (Fig. 1A). Ignoring the few late-season catches of moths in 1998 and 1999 as resulting from outside sources like wooden apple bins (SIR Programme, unpublished data), an apparent disappearance of second-generation codling moths by 1997 (Fig. 1A), combined with maintenance of S:W moth ratios greater than 40:1 during weeks 9 - 16 throughout 1996 - 1997 (Table 2), supports the view that it is possible to eliminate summer populations of codling moths within three years in localized areas. The difficulty comes in separating the cause(s) of this population decline. It seems likely that intensive insecticide application during first generation (Bloem and Bloem 2000) was critical in achieving this result. Insecticides applied against first-generation larvae will undoubtedly reduce resulting adult populations in second generation, especially when this is done on an area-wide basis as it was during this study. The potential for immigration of background wild populations was probably greatly reduced by this area-wide approach to spraying and other clean-up procedures. The effect of these insecticides is probably the reason why traps in conventional insecticide-treated orchards revealed little or no second-generation wild moth flight activity in 1995 and 1996, but in pheromone-treated orchards there was a relatively large second-generation catch in 1995 (Fig. 1A-B). It seems difficult to argue that observed reductions in second-generation wild moths (Fig. 1A) would have more to do with suboptimal S:W ratios in spring, than with insecticide applications. Likewise, providing evidence that large S:W ratios during summers of 1997 - 1999 had more to do with increasing catches of sterile moths (Fig. 2), than it did with the absence of a second-generation of wild moths (Fig. 1A), is difficult. This being the case, it may make more economic sense to apply insecticides during first generation and only release moths during the second generation.

In spite of extensive spraying, five years of moth release (1994 - 1998), and S:W ratios during second-generation that were always above 40:1 in 1997 and 1998 (Table 2), first-generation moths persisted into 1999 in the insecticide-treated orchards. These data are similar to results reported for Zone 1 as a whole, where average trap captures for all orchards showed great reductions in the second generation in 1995, and persistence of a small first generation through 1997 (Bloem and Bloem 2000). Further evidence of this persistence came from 1500 Zone-1 orchards sampled in 1999 using cardboard tree bands (Judd *et al.* 1997), which found that 15% still had overwintering codling moth larval populations going into 2000 (HMAT, unpublished SIR Programme data). It appears a small portion of the larval population arising from first-generation mating can escape control by insecticides in spring and sterile insect technique in summer. We hypothesize that early-emerging univoltine larvae not killed by insecticides in spring, and diapausing in summer because they are univoltine, could potentially escape both controls. Studies of the degree to which diapause and voltinism may affect predictions of eradication or long-term management of codling moth populations are warranted, especially as efforts to release sterile insects move further north in BC where a greater degree of early diapause is anticipated.

Predictions of eradication of codling moth from Zone 1 by 1996 (Dyck *et al.* 1993) and subsequent predictions by 1999

(Bloem and Bloem 2000) were not realized. Our data suggest that low S:W overflooding ratios during spring may have contributed to a slow rate of population decline. Several studies have emphasized the point that sterile males have their greatest impact on reproduction of codling moth in first generation (Proverbs *et al.* 1966; Proverbs 1971). In studies where codling moth population reduction has clearly been demonstrated using sterile insect technique (Proverbs *et al.* 1966, 1977; Proverbs *et al.* 1982), the authors report it was rare for S:W ratios to fall below 40:1, at anytime during the season. Inadequate spring-time ratios in the BC SIR Programme, means control is largely exerted against one generation each year. Controlling three generations instead of six over a three-year period, likely doubles the time required for eradication from 3 - 6 years, which seems consistent with reported progress (Bloem and Bloem 2000). Within-season and between-orchard variation in S:W ratios was not revealed by average yearly overflooding ratios (Table 1), a coarse-grained statistic often used in sterile insect programmes. These summary type ratios are artificially inflated by summing catches of sterile moths during periods of the season when there are no wilds, thus giving a misleading impression of the control effort being achieved. This type of reporting may not be applicable in an area-wide programme where there are many, small, noncontiguous orchards and wild populations to be eliminated.

If eradication were to be a goal of any SIR programme against codling moth, then efforts must include continued use of insecticides in spring, or reduction of early-season reproduction by means other than sterile moths. In choosing supplemental controls it must be noted that while applying insecticides during first generation obviously increases larval mortality, it also kills sterile moths, or potentially impairs their pheromone response (Linn and Roelofs 1984; Haynes and Baker 1985). Biological insecticides like Virosoft-CP4®, a commercial granulovirus product may be useful because they act on larvae and not adult moths. Our results suggest application of pheromone-disruption treatments against first-generation moths may also be very useful in augmenting control by sterile insects, especially in organic orchards.

Although codling moth was not eradicated in BC and progress was slower and more costly than anticipated, it was reduced to sub-economic levels in most Zone-1 orchards by 2001. Reliance on sterile insect technology seems capable of keeping damage below economic levels but there is some question about financial sustainability (Dendy *et al.* 2001). When much of the expenditure in a typical SIR programme is on rearing and releasing moths, the moths must be used effectively. In our analysis, we have focussed on a ratio of 40:1, because all previous work suggested this was necessary for eradication. However, it appears that ratios less than 40:1 can stabilize but not eliminate populations, and provide some suppression rather than eradication. The key question for management then becomes what ratios are acceptable in that new context.

An analysis of the SIR Programme trapping data from 1994 - 2004, on an individual orchard basis, within a spatial context, may provide insight on the S:W ratios providing suppression. If historic operational S:W overflooding ratios were correlated with damage data, analysis may reveal why the programme has worked in some orchards and areas, and not in others. Such analysis may also provide a more realistic appraisal of any codling moth SIR programme and would compliment the detailed observations we have made on a subset of orchards. This type of analysis will also be useful in planning the best use of sterile codling moths in BC, and other parts of the world where this technique is currently being considered.

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Use of Ethyl and Propyl (*E,Z*)-2,4-decadienoates in Codling Moth Management: Improved Monitoring in Bartlett Pear with High Dose Lures

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ABSTRACT

The propyl and ethyl esters of (*E,Z*)-2,4-decadienoic acid were evaluated in gray halobutyl septa as kairomone lures for both sexes of codling moth, *Cydia pomonella* (L.). All studies were conducted in 'Bartlett' pear orchards with moderate to high codling moth adult population densities and treated with sex pheromone dispensers for mating disruption. Variable results were obtained with kairomone loading experiments. Increasing the lure loading to 40.0 mg of either the ethyl or propyl ester significantly increased male and total moth catch in separate experiments. However, in other tests with the ethyl ester no difference was found in total moth catch in traps baited with 0.1, 1.0, 3.0 or 10.0 mg versus 40.0 mg lures. The 40.0 mg ethyl and propyl ester lures were both more effective than a 3.0 mg ethyl ester lure and comparable to a sex pheromone lure in detecting the beginning of codling moth flight in the spring generation. No difference was found in moth catch between 40.0 mg propyl and ethyl ester lures. Significantly more females were caught in traps baited with 1.0 – 10.0 mg than with 1.0 – 100.0 µg lures loaded with the ethyl ester. In general, kairomone lures caught significantly fewer moths than sex pheromone lures.

Key Words: *Cydia pomonella*, monitoring, kairomone, trapping

INTRODUCTION

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Torticidae), is a worldwide, key internal pest of pear, *Pyrus communis* L., and adult populations in commercial orchards are typically monitored with traps baited with sex pheromone lures (Riedl *et al.* 1986). Reliable adult monitoring is especially critical in orchards where codling moth is managed with applications of its sex pheromone for mating disruption (Knight 2002a). The sex pheromone treatment disrupts the attractiveness of the sex pheromone-baited trap, and traps can fail to alert pest managers of potential problems. Efforts to improve the usefulness of traps in sex pheromone-treated orchards have included the use of high-dose lures (Charmillot 1990) and an increased density of traps (Gut and Brunner 1996).

Identification of ethyl (*E,Z*)-2,4-decadienoate as a potent kairomone attractant for codling moth adults and larvae has allowed the development of several new approaches to successfully monitor and manage this pest (Knight and Light 2001; Light *et al.* 2001). Ethyl (*E,Z*)-2,4-decadienoate is attractive to both sexes of codling moth, and traps can be used to assess the timing of female emergence and activity, as well as mating status. In addition, the attractiveness of the lure is not strongly affected by the application of sex pheromones (Light *et al.* 2001), and the lure can improve the prediction of local pest population densities (Knight 2002b).

Ethyl (*E,Z*)-2,4-decadienoate is a major volatile of ripe pear (Jennings *et al.* 1964), and is not found in immature fruit or pear-

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leaf volatiles (Miller *et al.* 1989, Scutareanu *et al.* 1997). An initial evaluation suggested that ethyl (*E,Z*)-2,4-decadienoate might not be an effective attractant for codling moth adults in pear orchards (Light *et al.* 2001). However, this conclusion was based on data collected with 1.0 mg lures placed in three conventional 'Bartlett' orchards with high levels of codling moth (> 3 moths trapped per day with a sex pheromone lure and detectable levels of fruit injury). The relatively low attractiveness of the synthetic ethyl (*E,Z*)-2,4-decadienoate lure in these pear orchards was hypothesized to be due to olfactory "masking" of the lure by natural sources of ethyl (*E,Z*)-2,4-decadienoate released either from injured or ripening fruits or by competing host volatiles (Light *et al.*, 2001). A second study found that lures loaded with 3.0 mg of ethyl (*E,Z*)-2,4-decadienoate were similar or more attractive than sex pheromone lures in four cultivars of pear when orchards were treated with sex pheromones (Knight *et al.* 2005). Ethyl (*E,Z*)-2,4-decadienoate lures performed similarly early and late in the season relative to a sex pheromone lure in these cultivars suggesting that maturation of fruit was not an important factor affecting lure attractiveness. Ethyl (*E,Z*)-2,4-decadienoate lures again performed poorly only in 'Bartlett' pear orchards with high population densities of codling moth that were defined by catches in sex pheromone-baited traps exceeding 20 moths per season and the presence of fruit injury (Knight *et al.* 2005).

Improvements in developing a more attractive kairomone lure for codling moth in pear may involve optimizing its emission rate and/or the use of alternative attractants. Light *et al.* (2001) reported that ethyl (*E,Z*)-2,4-decadienoate loaded into gray halobutyl lures was attractive at doses > 10.0 μg, and the highest moth catches in walnut orchards occurred with 10.0 mg lures. Studies testing doses of ethyl (*E,Z*)-2,4-decadienoate > 3.0 mg in pear have not been reported. Among the 23 volatile blends tested by Light *et al.* (2001), only the blend of methyl and ethyl esters of ten-carbon acids were attractive to adult codling moth. Within this group, methyl (*E,Z*)-2,4-decadienoate was found to be somewhat attractive in both apple and walnut orchards. This compound was also attractive to neonate codling moth (Knight and Light 2001). Unfortunately, the methyl ester is another component of ripe pears (Heinz *et al.* 1966, Shiota 1990), and its attractiveness to codling moth would also likely be influenced by competition with volatile fruit odors within the orchard.

An alternative approach in developing an improved kairomone may be to utilize a related volatile that is not present in pear orchards. Propyl (*E,Z*)-2,4-decadienoate has been identified from pear by GLC analysis following an isopentane extraction of ripe 'Bartlett' puree (Creveling and Jennings 1970), but not from volatile headspace collections of intact fruit (Light *et al.* 2001) or codling moth-injured fruit (D. M. L., unpublished data). GC-EAD antennal response to a synthetic homologous series of decadienoic esters found that codling moth's antennal receptivity is stimulated by the ethyl ester, with both the methyl- and propyl-esters eliciting a smaller depolarization response (D. M. L., unpublished data). The behavioral effects elicited in codling moth by propyl (*E,Z*)-2,4-decadienoate are unreported. Herein, we report studies optimizing the dose of ethyl and propyl (*E,Z*)-2,4-decadienoate in separate lures for monitoring adult codling moth in 'Bartlett' pear.

MATERIALS AND METHODS

Field test protocol. Field trials were conducted in 1999, 2001, and 2002 to evaluate the attractiveness of lures loaded with different doses of ethyl and propyl (*E,Z*)-2,4-decadienoate versus sex pheromone lures (L2™ and Megalure™, Trécé Inc., Salinas, California [CA]). The ethyl (93.7% A.I.) and propyl esters (94.7%

A.I.) were synthesized by Trécé Inc. and loaded in gray halobutyl elastomer septa. All studies were conducted in 'Bartlett' pear orchards located near Wapato, Washington (WA) and treated with 700 – 1,000 Isomate-C PLUS sex pheromone dispensers (Pacific Biocontrol, Vancouver, WA) per ha. All studies were conducted with diamond-shaped sticky traps (Pherocon IIB, Trécé Inc., Salinas, CA) attached to poles placed in the upper third of the trees' canopy. Traps were placed 30 – 50 m apart within each orchard. Traps were checked weekly and replaced when needed. Moths were sexed in all studies.

Optimizing the lure dose of ethyl (*E*,*Z*)-2,4-decadienoate. Two experiments were conducted in 1999 to examine various doses of ethyl ester. Experiment 1 compared the attractiveness of lures loaded with the ethyl ester at doses of 1.0, 10.0, and 100.0 µg and 1.0, 3.0, and 10.0 mg versus the L2™ sex pheromone lure. Eight replicates of each lure dose were used. Traps were randomized, placed in the orchard on 21 May, and checked weekly until 28 June. Traps were not rotated in this study. Experiment 2 evaluated a smaller subset of lure doses of the ethyl ester: 1.0, 10.0, and 40.0 mg, versus the Megalure™. Nine replicates of each lure type were randomized in the field on 30 June, and traps were checked and rotated weekly until 27 July.

Optimizing the lure dose of propyl (*E*,*Z*)-2,4-decadienoate. An experiment was conducted in 2001 to compare the attractiveness of the propyl ester at lure doses of 1.0, 3.0. 10.0 and 40.0 mg versus the standard 3.0 mg ethyl ester lure. Ten replicates of each lure dose and type were randomly placed within a single orchard on 9 May and checked weekly until 5 July. Traps were rotated within the orchard each week.

Comparison of kairomone and sex pheromone lures. Two experiments were conducted to compare the attractiveness of lures loaded with 3.0 or 40.0 mg ethyl ester, a lure loaded with 40.0 mg propyl ester, and the commercial high-dose sex pheromone lure, Megalure™. The first experiment was conducted during 2001 in ten orchards. Traps were placed in each orchard on 3 July and checked and rotated each week until 15 August. The second experiment was conducted in 2002 in the same ten orchards. Traps were placed in the orchards on 22 April and checked and rotated weekly until 1 July.

Data analysis. Significant differences in the cumulative moth catch in traps baited with each lure over the specified time interval were determined with one way analysis of variance (ANOVA), $P < 0.05$ (Analytical Software 2000). Count data were transformed prior to analysis with square root (x + 0.01). Differences in the detection of first moth flight among lures were determined by ANOVA with data collected in 2002. Means were separated with Fisher's least significant difference test within all significant ANOVA's.

RESULTS

Optimizing the lure dose of ethyl (*E*,*Z*)-2,4-decadienoate. Significant differences were found among the various doses of the ethyl ester and the sex pheromone lure in the cumulative catch of male ($F = 20.38$; df = 6, 49; $P < 0.001$) and total moths ($F = 16.09$; df = 6, 49; $P < 0.001$) in experiment 1 (Table 1). The sex pheromone lure caught significantly more males and total moths than any of the ethyl ester lures. Traps baited with ethyl ester lures loaded with 1.0, 3.0, and 10.0 mg caught significantly more males than traps baited with 1.0 and 10.0 µg lures and more total moths than traps baited with the 1.0 µg lure. Significant differences were found in the catch of female moths among the kairomone lures ($F = 7.19$; df = 5, 42; $P < 0.001$) (Table 1). Lures loaded with 1.0 – 10.0 mg ethyl ester caught significantly more female moths than lures loaded with 1.0, 10.0, and 100.0 µg (Table 1).

Table 1.
Attractiveness of ethyl (*E,Z*)-2,4-decadienoate (Et-*E,Z*-DD) lures compared with sex pheromone lures in 'Bartlett' pear orchards, 1999.

		Mean cumulative capture ± SE of codling moth per trap[1]		
Exp.[2]	Lure, loading	Males	Females[3]	Total
1	Et-*E,Z*-DD, 1.0 µg	0.50 ± 0.38d	0.38 ± 0.18b	0.88 ± 0.52d
	Et-*E,Z*-DD, 10.0 µg	1.25 ± 0.59cd	0.75 ± 0.31b	2.00 ± 0.85cd
	Et-*E,Z*-DD, 100.0 µg	3.13 ± 0.88bc	1.00 ± 0.50b	4.13 ± 0.99bcd
	Et-*E,Z*-DD, 1.0 mg	4.50 ± 0.93b	3.13 ± 1.01a	7.63 ± 1.61bc
	Et-*E,Z*-DD, 3.0 mg	5.25 ± 1.10b	3.25 ± 0.75a	8.50 ± 1.46b
	Et-*E,Z*-DD, 10.0 mg	5.50 ± 0.99b	4.75 ± 0.90a	10.25 ± 1.71b
	Sex pheromone, N.A. [3]	28.00 ± 5.20a	-	28.00 ± 5.20a
2	Et-*E,Z*-DD, 1.0 mg	2.33 ± 0.80c	0.89 ± 0.54a	3.22 ± 1.01c
	Et-*E,Z*-DD, 10.0 mg	1.78 ± 0.49c	0.89 ± 0.35a	2.67 ± 0.82c
	Et-*E,Z*-DD, 40.0 mg	8.33 ± 1.73b	1.00 ± 0.47a	9.33 ± 2.13b
	Sex Pheromone, N.A. [3]	21.78 ± 4.98a	-	21.78 ± 4.98a

[1] Column means within each experiment followed by a different letter are significantly different at $P < 0.05$, Fisher's LSD.
[2] Experiment 1 was conducted from 21 May –22 June and experiment 2 was conducted from 30 June – 27 July 1999.
[3] Not available. Proprietary loadings in the L2™ and Megalure™ sex pheromone lures used in experiments 1 and 2, respectively have not been published (Trécé Inc., Salinas, CA).

Significant differences in the catch of male ($F = 12.08$; df = 3, 32; $P < 0.0001$) and total moths ($F = 15.37$; df = 3, 32; $P < 0.0001$) among the ethyl ester and sex pheromone lures were also found in experiment 2 (Table 1). The sex pheromone lure caught significantly more males and total moths than any of the three doses of the ethyl ester tested. The 40.0 mg ethyl ester lure caught significantly more males and total moths than the 1.0 and 10.0 mg lures. No significant differences in the catch of female moths occurred among doses of the ethyl ester in this experiment ($F = 0.02$; df = 2, 24; $P = 0.98$).

Optimizing the lure dose of propyl (*E,Z*)-2,4-decadienoate. Significant differences were found in the mean captures of males ($F = 3.91$; df = 4, 45; $P < 0.01$) and total moths ($F = 3.23$; df = 4, 45; $P < 0.05$) among different doses of the propyl ester and the 3.0 mg ethyl ester lure (Table 2). The highest dose of the propyl ester (40.0 mg) caught significantly more males and total moths than the other lures. No difference was found among lures in the catch of female codling moth ($F = 2.29$; df = 4, 45; $P = 0.07$).

Comparison of kairomone and sex pheromone lures. During the first generation flight in 2002 there was no difference in moth catch among the sex pheromone and kairomone lures ($F = 0.97$; df = 3, 36; $P = 0.42$). However, there was a significant difference among lures in the first detection of moth flight ($F = 4.39$; df = 3, 36; $P < 0.01$). The first adult codling moth caught by the 3.0 mg ethyl ester lure was on average nearly 2 wk later than the other lures (Table 3). The high-dose kairomone and sex pheromone lures did not differ in their detection of the start of codling

Table 2.
Evaluation of the attractiveness of ethyl (*E,Z*)-2,4-decadienoate (Et-*E,Z*-DD) and propyl (*E,Z*)-2,4-decadienoate (Pr-*E,Z*-DD) lures in ten Bartlett pear orchards from 9 May to 5 July 2001.

		Mean cumulative capture ± SE of codling moth per trap[1]		
Lure	Loading (mg)	Males	Females	Total
Pr-*E,Z*-DD	1.0	2.3 ± 0.6b	0.8 ± 0.3a	3.1 ± 0.6b
Pr-*E,Z*-DD	3.0	2.6 ± 0.6b	1.0 ± 0.3a	3.6 ± 0.8b
Pr-*E,Z*-DD	10.0	2.4 ± 0.4b	0.2 ± 0.1a	2.6 ± 0.4b
Pr-*E,Z*-DD	40.0	5.4 ± 0.9a	0.6 ± 0.3a	6.0 ± 1.0a
Et-*E,Z*-DD	3.0	3.5 ± 0.7b	0.2 ± 0.1a	3.7 ± 0.6b

[1] Column means followed by a different letter are significantly different at $P < 0.05$, Fisher's LSD.

Table 3.
The effectiveness of the ethyl (*E,Z*)-2,4-decadienoate (Et-*E,Z*-DD), propyl (*E,Z*)-2,4-decadienoate (Pr-*E,Z*-DD), and sex pheromone lures in monitoring first generation codling moth in ten 'Bartlett' pear orchards treated with sex pheromone, 22 April to 1 July 2002.

Lure	Loading (mg)	Mean ± SE cumulative moth catch per trap[1]	Mean ± SE weeks to first moth catch
Pr-*E,Z*-DD	40.0	9.4 ± 1.7a	3.1 ± 0.5a
Et-*E,Z*-DD	3.0	14.2 ± 3.6a	4.8 ± 0.2b
Et-*E,Z*-DD	40.0	15.4 ± 3.7a	2.2 ± 0.1a
Sex pheromone	N.A. [2]	24.8 ± 12.0a	3.0 ± 0.8a

[1] Column means followed by a different letter are significantly different at $P < 0.05$, Fisher's LSD.
[2] Not available. Proprietary loading in the Megalure™ high-dose sex pheromone lure has not been published (Trécé Inc., Salinas, CA).

moth's spring flight. A significant difference in the capture of codling moth adults was found among lures during the second generation in 2001 ($F = 4.06$; df = 3, 36; $P < 0.05$). The sex pheromone lure caught 4- to 13-times more moths than the various kairomone lures (Table 4). No differences in male, female, and total moth catch were found among kairomone lures in this test.

DISCUSSION

The hypothesis and rationale for the synthesis and testing of the un-natural synthetic propyl ester were the potential for its greater detectability, attractiveness, or capture activity in orchards where high levels of pear fruit damage are causing the precocious liberation of large, perhaps masking, amounts of the natural methyl and ethyl esters. However, our results with the propyl ester lures were inconsistent. The 40.0 mg propyl ester lure caught significantly more total moths and males than similar lures loaded with 1.0 – 10.0 mg and more than the 3.0 mg ethyl lure during May and

Table 4.
Evaluation of the attractiveness of the ethyl (*E,Z*)-2,4-decadienoate (Et-*E,Z*-DD), propyl (*E,Z*)-2,4-decadienoate (Pr-*E,Z*-DD), and a commercial sex pheromone lure in monitoring second generation codling moth flight in ten 'Bartlett' pear orchards treated with sex pheromone, 3 July to 15 August 2001.

		Mean ± SE cumulative moth catch per trap[1]		
Lure	Dose (mg)	Males	Females	Total
Pr-*E,Z*-DD	40.0	3.5 ± 1.9b	3.5 ± 1.9a	7.0 ± 4.5b
Et-*E,Z*-DD	3.0	3.0 ± 1.0b	3.1 ± 1.8a	6.1 ± 2.7b
Et-*E,Z*-DD	40.0	2.0 ± 0.5b	1.4 ± 0.6a	3.4 ± 0.9b
Sex pheromone	N.A.[2]	26.9 ± 9.3a	-	26.9 ± 9.3a

[1] Column means followed by a different letter are significantly different at $P < 0.05$, Fisher's LSD.
[2] Not available. Proprietary loading in the Megalure ™ high-dose sex pheromone lure has not been published (Trécé Inc., Salinas, CA).

June in 2001, yet from July to mid August in 2001 and from late April through June in 2002 traps baited with the 40.0 mg propyl lure did not catch more moths than the 3.0 mg ethyl ester lure. Thus no clear advantage in using the propyl ester versus the ethyl ester was demonstrated in these studies.

The effect of increasing the loading rate of the ethyl ester was also inconsistent among experiments. No significant difference in moth catch was found in traps baited with 1.0 – 10.0 mg ethyl ester in late May to June in 1999, but the 40.0 mg lure caught significantly more total moths and males than either the 1.0 or 10.0 mg lure during July. However, from July to mid August in 2001 and from late April through June in 2002 no difference in moth catch occurred in traps baited with either a 3.0 or 40.0 mg ethyl ester lure.

The 40.0 mg kairomone lures, however, were effective in detecting the beginning of the first generation moth flight in pear, whereas moth catch in traps baited with the 3.0 mg ethyl ester lure was delayed. Similar patterns of delayed first moth catch have been found with 3.0 mg ethyl ester lures in apple orchards (A.L.K., unpublished data), but not in walnut (Light *et al.* 2001). Accurate detection of the start of moth flight ("Biofix") is widely used to predict the start of egg hatch and in timing insecticide applications (Riedl *et al.* 1976). Thus, the 40.0 mg ethyl ester lure may be an improved kairomone lure to monitor early-season codling moth flight activity.

Traps baited with either kairomone were not as effective as traps baited with sex pheromone in capturing codling moth in all but one study (early season 2002 trial). This negative result was similar to other data collected with the 3.0 mg ethyl ester lure from 'Bartlett' orchards in the same fruit-growing region of WA (Knight *et al.* 2005). The relatively poor performance of the ethyl ester lure in both WA studies is in contrast to its higher performance than sex pheromone lures in CA 'Bartlett' orchards (Zoller and Zoller 2003). A major difference between the published WA and CA studies is the population densities of codling moth within the monitored orchards. Mean moth catch in sex pheromone-baited traps was >80 moths per trap per season in WA 'Bartlett' orchards (Knight *et al.* 2005) and >20 moths per trap over the one- to two-month studies reported here. Codling moth fruit injury occurred at levels >10.0% in many of these orchards. In comparison, mean cumulative moth catch in the sex pheromone-baited traps was 2.7 per season in CA 'Bartlett' orchards and fruit injury was <0.5%

(Zoller and Zoller 2003). A similar reduction in the attractiveness of the ethyl ester was found in the second generation of codling moth for apple orchards with high levels of fruit injury (Light *et al.* 2001). Additional studies are needed to refine under which field conditions the pear esters are likely to perform well, i.e. crop, cultivar, season, crop load, fruit maturity, and percent injured fruit (Knight 2002b). Comparing the statistical correlations of moth catch in sex pheromone- and kairomone-baited traps with egg density or levels of fruit injury versus the absolute counts would likely be more informative in developing an improved monitoring program for codling moth.

Similarly, interpretation of the numbers and timing of female moth capture is a unique feature of kairomone-baited traps that has not been fully utilized. Traps baited with ethyl ester typically catch from 40 – 60% female codling moth (Light *et al.* 2001), and similar data were reported across four pear cultivars in orchards with both low and high moth population densities (Knight *et al.* 2005). In our current study, female moths were caught in traps baited with either ester and across all lure-loading rates. Significantly more female moths were caught in traps baited with $\geq$1.0 mg ethyl ester than at the lower rates tested and no additional increase was found with lure loadings up to 40.0 mg. Results with the propyl ester were similar and no differences in female catch were noted between 40.0 mg ethyl and propyl lures. Establishing moth catch thresholds based on female codling moth density could be a useful approach to improve management of this pest, especially in sex pheromone-treated orchards (Knight 2002b). In addition, initiating predictive timing models of egg hatch based on a "Biofix" of first female moth catch could improve current models. Incorporating these kairomone-baited traps in the integrated management of codling moth will require additional testing and refinement.

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Use of Ethyl (*E,Z*)-2,4-decadienoate in Codling Moth Management: Stimulation of Oviposition

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ABSTRACT

The effect of the pear volatile, ethyl (*E,Z)*-2,4-decadienoate (Et-*E,Z*-DD), on oviposition by codling moth, *Cydia pomonella* (L.), was evaluated in a series of choice and no-choice laboratory experiments and in subsequent field tests conducted in apple and walnut. Gray halobutyl elastomer septa loaded with 1.0 and 100.0 µg Et-*E,Z*-DD significantly increased the numbers of eggs laid by a laboratory population in 96 h no-choice assays by 2-fold. In addition, the number of eggs laid near the Et-*E,Z*-DD versus a solvent blank dispenser was significantly higher in choice bioassays across a similar range of septa loadings. Oviposition rates by a field-collected post-diapause strain of codling moth were significantly increased by the addition of a 1.0-µg septa versus a solvent blank dispenser in a no-choice bioassay. Field trials were conducted in apple and walnut to develop an artificial egg trap baited with Et-*E,Z*-DD to monitor codling moth oviposition. Septa loaded with 0.1 to 10.0 mg did not significantly increase oviposition versus solvent blank dispensers on a Mylar plastic collar trap or on the adjacent leaves and fruit in apple. Significantly more eggs were laid on the fruit and foliage than on the plastic collar. No eggs were deposited on non-bearing apple shoots baited with 0.1 – 40.0 mg Et-*E,Z*-DD septa. Similarly, no eggs were deposited on cylindrical wax paper-covered plastic traps baited with 10.0 µg to 1.0 mg Et-*E,Z*-DD septa in walnut orchards. The potential of Et-*E,Z*-DD to monitor codling moth's oviposition in the field, stimulate oviposition by field-collected strains under laboratory conditions, and to improve pest control by disrupting host location are discussed.

Key Words: *Cydia pomonella*, oviposition, kairomone, phenology, egg trap

INTRODUCTION

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is typically monitored in fruit and nut orchards with sex pheromone-baited traps. The numbers and timing of male moths captured in these traps is used to infer the density of female moths and timing of oviposition (Riedl and Croft 1974, Riedl *et al.* 1976). The development of an effective, inexpensive tool to directly monitor the density of codling moth females and/or oviposition could improve its management. The sesquiterpene, (*E,E*)-α-farnesene (*E,E*-αF), a major constituent of apple fruit and leaf odors, was identified as a key adult and larval attractant for codling moth (Sutherland *et al.* 1974) and was shown to stimulate oviposition in both choice and no-choice laboratory bioassays (Wearing and Hutchins 1973). Unfortunately, *E,E*-αF is unstable in the presence of oxygen and has a very short residual activity (Anet 1969). Field trials evaluating the stimulatory effect of E,E-αF on codling moth oviposition have not been reported.

Direct monitoring of codling moth egg density in orchards through foliage and fruit sampling is labor intensive and often ineffective due to the relatively low population density of this pest in commercial orchards (Elkins 2002). A novel approach to monitor codling moth egg density in

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California pear orchards has recently been reported that uses the artificially cutting or damaging of fruit (Zoller and Zoller 2001). Pear clusters containing one cut-fruit (2.0-3.0 cm wedge cut 0.5-1.0 cm deep) were 95-times more likely to contain a codling moth egg than normal fruit clusters. Yet, a number of factors increase the variability of this approach including cultivar and days before harvest, *i.e.*, Bosc pear clusters were more attractive than Bartlett; and the attractiveness of cut- versus uncut-fruit was only significantly different during the month before harvest (Zoller 2001). Unfortunately, the incidence of eggs among the cut-fruit cluster samples in commercial pear orchards was low (< 1.0%), and the utility of this labor-intensive approach for improving the management of codling moth is unclear (Zoller and Zoller 2001).

Light *et al.* (2001) and Knight and Light (2001) found that ethyl (*E,Z)*-2,4-decadienoate (Et-*E,Z*-DD), a principal odorant from ripe pear (Jennings *et al.* 1964), was a potent adult and larval kairomonal attractant for codling moth. Et-*E,Z*-DD appears to be chemically more stable than *E,E*-αF and can be loaded in septa to provide long-lasting, effective monitoring of adult populations (Light *et al.* 2001; Knight *et al.* 2005). The potential effects of Et-*E,Z*-DD on oviposition of codling moth have not been reported. Herein, we report on the effects of Et-*E,Z*-DD on codling moth oviposition under laboratory conditions and evaluate its use as a lure to monitor egg laying in apple and walnut orchards.

MATERIALS AND METHODS

Choice and no-choice laboratory experiments. Moths 24-48 hr old were obtained from a laboratory colony reared on artificial diet (Toba and Howell 1991) at the U.S.D.A. Yakima Agricultural Research Laboratory in Wapato, WA. Individual virgin female moths were paired with two male moths for 24 h at 25 °C, > 40% R.H., and a 16:8 L:D photoperiod. Females were then placed in screened (3 x 3 mm) cylindrical cages (11 cm long and 9 cm diameter). Both ends of each cage were capped with a plastic cover lined with wax paper. Gray halobutyl elastomer septa (No. 1888, size No. 1,West Co., Phoenixville. PA) were pinned to the wax paper at each end of the cage. A 10.0% honey water wick was inserted into the middle of each cage.

Choice and no-choice tests were conducted with septa loaded with 1.0 and 100.0 µg Et-*E,Z*-DD (93.7% A.I., Trécé Inc., Adair, OK) versus septa treated only with the solvent, dichloromethane as a blank. In choice tests an Et-*E,Z*-DD-loaded septa and a solvent blank septa were pinned to the wax paper at opposite ends of the cage. Identical lures were pinned at each end of the cage in the no-choice tests. Tests were run for 96 h inside greenhouses maintained between 20 – 25 °C. Cages were spaced > 5 m apart and no more than four replicates were run on each of six dates. An additional no-choice test was conducted with 1.0 µg Et-*E,Z*-DD septa versus a solvent control septa using moths reared from an overwintering population collected as larvae the previous season in corrugated bands attached to apple trees. Larvae had been maintained under diapause conditions (2 °C and total darkness) for five months and were allowed to emerge in rooms maintained at 25 °C, 16:8 L:D and > 45% R.H. The numbers of eggs laid on each wax paper cap were counted in all experiments and females were dissected to determine their mating status. Only data from mated females were included in the statistical analyses (15 – 20 replicates per comparison).

Development of an egg trap. Studies were conducted in a greenhouse to develop a suitable substrate for an artificial egg trap. The suitability of waxed cardboard and Mylar plastic as sites of codling moth oviposition were compared with natural apple leaves. Three mated females (< 72 h old) were placed inside of screened cages

with a 1.2 m potted apple seedling. Artificial substrates were cut to mimic a typical leaf shape (19.8 cm^2) and were clipped to the main stem of the plant. In the first test we compared oviposition on the plastic versus the waxed cardboard substrate. The height of each type of artificial leaf was alternated on the seedling at 0.7 and 1.0 m, and six replicate cages were evaluated from 14 – 19 March 2001. In a second test, we compared oviposition on a selected apple leaf on the seedling versus a similar-sized plastic leaf. Both the natural and artificial leaves were positioned on the seedling at a height of 1.0 m and on opposite sides of the shoot. Twenty-four cage replicates of this test were conducted between 23 March and 26 April 2001. The number of eggs on each artificial and natural apple leaf was counted after 5 d.

Field evaluation of egg traps in apple. Studies were conducted in an unsprayed 'Red Delicious' apple orchard situated near Parker Heights, WA from 18 – 29 May 2001. Square plastic sheets (0.1 m^2) were slit and folded as collars around fruit and foliage clusters and baited with either 0.1, 1.0, or 10.0 mg Et-*E*,*Z*-DD lures or an unbaited solvent-only lure (nine replicates per treatment). Baited trees were spaced > 20 m apart and traps were placed in the upper third of the canopy. Fruit monitoring indicated that no fruit injury was present in the orchard when the experiment was started. Fruit and leaf clusters were removed after 7 d and plastic collars, fruits and leaves were then examined in the laboratory for codling moth eggs and injury. A second test was conducted from 5 – 11 June 2001 in another unsprayed 'Red Delicious' apple orchard situated near Moxee, WA using the plastic collar trap baited with either 3.0 mg Et-*E*,*Z*-DD or solvent-only lures. Twenty replicates of each lure type were placed on shoots with and without fruit. Only uninjured fruit clusters were used in this study. Collars, foliage, and fruit were examined for eggs after 6 d. Eggs in both studies were categorized into developmental stages based on their morphology (Richardson *et al.* 1982). Only 'white' eggs and 'red ring' eggs characterized as having only one visible stemmata (< stage 11) were scored as having been laid during each test.

A third apple trial was conducted during 2002 in the Moxee apple orchard to examine if oviposition could be stimulated to occur on non-bearing shoots baited with Et-*E*,*Z*-DD lures. Septa loaded with either 1.0, 3.0, 10.0, 20.0, or 40.0 mg Et-*E*,*Z*-DD and solvent-only septa (10 replicates per treatment) were pinned to non-bearing shoots on 15 June. One septum was attached per tree and baited-trees were spaced 5 – 6 m apart. Five replicates of each lure loading were collected after 10 d and the rest were collected after 20 d. All shoots were examined for hatched and unhatched eggs. Two Et-*E*,*Z*-DD-baited sticky delta-shaped traps (Trécé Inc., Adair, OK) were used to monitor the activity of female codling moths in this orchard during this test. In addition, 30 randomly selected fruit from 20 trees situated within 100 m of the center of the study site were inspected for codling moth injury at the end of this test to assess pest pressure.

Field evaluation of egg traps in walnut. Studies were conducted from mid-June through September 2002 in a 'Chandler' walnut orchard (Dixon, CA) characterized as having a moderate codling moth population density based on early-season moth catches in sex pheromone-baited traps. An oviposition trap was constructed using a plastic sheet (21.0 x 28.0 cm) covered with wax paper on both sides. The plastic sheet was rolled into a 9 cm diameter tube, then secured and hung horizontally by a wire trap hanger. Septa impregnated with either 10.0, 100.0, or 1,000.0 µg Et-*E*,*Z*-DD or solvent-only were attached by a plastic septa holder from the center of the inner tube of the trap. Traps were hung in the upper third of the 8.0 m canopy and baited trees were separated by 80 m. Three replicate blocks were established within three orchards (9 replicates of each lure rate). Traps were checked weekly for eggs and septa were replaced every two weeks. In July, a 500-

nut sample was collected from the canopy in each replicate block and examined for codling moth injury.

Statistical analysis. The total number of eggs laid in cages baited either with Et-*E,Z*-DD or solvent-only septa in choice and non-choice tests were compared with paired and unpaired t tests, respectively, at a $P < 0.05$ level of significance (Analytical Software 2000). A paired t-test was used to compare the suitability of substrates for oviposition in the greenhouse studies. Analysis of variance (ANOVA) was used to evaluate the influence of lure loading rate on oviposition on Mylar traps and associated fruit and foliage in apple and to compare oviposition on the Mylar collar versus fruit and foliage. Mean separation following a significant ANOVA was determined with a LSD test, $P < 0.05$.

RESULTS

Choice and no-choice laboratory experiments. Septa loaded with Et-*E,Z*-DD significantly increased oviposition by a laboratory strain of codling moth in greenhouse tests in both choice and no-choice tests with lures baited with 1.0 and 100.0 µg Et-*E,Z*-DD versus solvent-only lures (Table 1). The mean number of eggs laid on wax paper next to the kairomone lure was 2-5 fold higher than next to a blank lure in choice-tests. In addition, approximately 2-fold more eggs were laid in cages baited with Et-*E,Z*-DD lures versus with solvent-only lures in no-choice tests. Field-collected codling moth females laid twice the number of eggs in cages baited with Et-*E,Z*-DD than in cages baited with the solvent control (Table 1).

Development of an egg trap. No difference was found in the number of eggs laid on the waxed cardboard leaf-model (mean $\pm$ SE = 9.7 $\pm$ 2.5) versus the plastic leaf-model (mean $\pm$ SE = 8.3 $\pm$ 2.3) (t = 0.39, df = 10, $P = 0.71$). The Mylar plastic leaf-model was chosen for subsequent field tests due to its greater stability when exposed to adverse field conditions, i.e. precipitation. However, significantly more eggs were deposited on the apple leaf (mean $\pm$ SE = 9.6 $\pm$ 2.4) versus the plastic leaf-models (mean $\pm$ SE = 4.3 $\pm$ 1.2) (t = -3.73, df = 46, $P < 0.001$).

Field evaluation of egg traps in apple. The mean $\pm$ SE number of fruit within the Mylar collar trap in the May 2001 test was 2.9 $\pm$ 0.3 and there was no significant difference in fruit density among lure treatments, $F = 0.35$; df = 3, 32; $P = 0.79$. The mean $\pm$ SE number of eggs that had already been deposited (> stage 11 plus hatched eggs) on the fruit and foliage surrounded by the Mylar collar was 1.3 $\pm$ 0.3 per cluster. There was no significant difference in the mean density of already deposited eggs among lure treatments in this test, $F = 0.93$; df = 3, 32; $P = 0.44$. Few new eggs (< stage 11) were laid on either the Mylar trap or the associated foliage and fruit during the May 2001 field experiment (Table 2). Total egg density on the Mylar, foliage, and fruit did not differ among lure types, $F = 0.87$; df = 3, 32, $P = 0.47$. Significantly more eggs were laid on the foliage and fruit than on the Mylar plastic collar across all lure loadings and the solvent blank, $F = 5.69$; df = 1, 70; $P < 0.05$. An average of 23% of the fruit within the Mylar collars were injured at the end of the experiment.

No eggs were deposited on Mylar collars or foliage in non-bearing shoots baited with either the 3.0 mg Et-*E,Z*-DD or a blank lure in the June 2001 test. Similarly, no eggs were deposited on the Mylar collars surrounding fruit. The mean $\pm$ SE number of fruit within the Mylar collar trap was 2.4 $\pm$ 0.1 and there was no significant difference in fruit density between lure treatments on fruit-bearing shoots, $F = 0.29$; df = 1, 38; $P = 0.59$. The mean $\pm$ SE number of eggs that had already been deposited (> stage 11 plus hatched eggs) on the fruit and foliage surrounded by the Mylar collar was 1.2 $\pm$ 0.3 per cluster. There was no significant difference in the mean density of already deposited eggs

Table 1.
The effects of ethyl (*E,Z*)-2,4-decadienoate loaded in gray halobutyl elastomer septa on egg laying of mated codling moth in choice and non-choice laboratory tests conducted for 96 h.

Test Type	Treatment	No. replicates	Mean ± SE no. eggs laid per cage	Statistical test [1] *P*-value
Choice	100.0 µg	18	21.0 ± 3.7	< 0.05
	Blank		11.6 ± 3.4	
Choice	1.0 µg	20	25.0 ± 5.2	< 0.001
	Blank		4.4 ± 1.4	
No-choice	100.0 µg	20	31.0 ± 3.4	< 0.001
	Blank		14.2 ± 2.8	
No-choice	1.0 µg	20	27.9 ± 5.2	< 0.05
	Blank		12.3 ± 3.0	
No-choice[2]	1.0 µg	15	13.1 ± 3.8	< 0.05
	Blank		5.7 ± 3.2	

[1] A paired and unpaired t-test were used to analyze data from the choice and no-choice tests, respectively.
[2] Field collected adults reared from pupae collected in corrugated bands placed in an unsprayed apple orchard.

Table 2.
Density of codling moth eggs deposited on Mylar traps and associated fruit and leaf clusters baited with ethyl (*E,Z*)-2,4-decadienoate or a solvent-only lure in an apple orchard during May (nine replicates per lure) and June (20 replicates per lure) 2001.

	Mean + SE no. eggs laid on		
Lure load (mg)	Mylar collar	Foliage and fruit	Both
	May 2001		
Solvent only	0.11 ± 0.11	0.22 ± 0.15	0.33 ± 0.17
0.1	0.00 ± 0.00	0.33 ± 0.17	0.33 ± 0.17
1.0	0.00 ± 0.00	1.11 ± 0.59	1.11 ± 0.59
10.0	0.22 ± 0.15	0.44 ± 0.34	0.67 ± 0.47
	June 2001		
Solvent only	0.00 ± 0.00	0.10 ± 0.10	0.10 ± 0.10
3.0	0.00 ± 0.00	0.35 ± 0.17	0.35 ± 0.17

between lure treatments on fruit-bearing shoots in this test, $F = 1.18$; df = 1, 38; $P = 0.28$. The number of eggs laid on foliage and fruit in collars baited with a 3.0 mg versus the solvent-only lure was not significant ($F = 1.66$; df = 1, 38; $P = 0.21$). An average of 11% of the fruit within Mylar collars were injured at the end of the experiment.

No eggs were deposited on non-bearing apple shoots baited with Et-*E,Z*-DD or the solvent control lures during the 2002 study. The mean ± SE capture of female codling moth in sticky traps baited with a Et-*E,Z*-DD lure was 0.3 ± 0.1 moths per night. Fruit injury by codling moth on a sample of trees within 100 m of the study site averaged 18.7% in mid July.

Field evaluation of egg traps in walnut. No eggs were laid on the wax paper-covered plastic tube traps placed in walnut orchards during the 14 wk study. How-

ever, the population of codling moth in this orchard was moderate to high. Nut damage in July averaged 3.0% in canopy samples. In addition, traps baited with both sex pheromone and Et-*E*,*Z*-DD lures caught a large number of moths, 3 – 6 moths per trap per night during the test. Codling moth adults were observed on several occasions resting on the plastic egg traps.

DISCUSSION

Our studies to develop an effective monitoring trap for codling moth oviposition with Et-*E*,*Z*-DD in apple and walnut were largely unsuccessful. Mylar and waxed paper were poor artificial substrates for oviposition by codling moth compared with fruit and foliage. Despite working in orchards with relatively very high population densities of codling moth (levels of fruit and nut injury $\geq$ 3.0%), rates of oviposition on fruit and leaves were low, and the addition of a Et-*E*,*Z*-DD lure did not significantly increase the number of eggs deposited. Baiting non-bearing shoots with Et-*E*,*Z*-DD lures did not stimulate oviposition.

Several factors likely contribute to the difficulty in developing an effective egg trap for codling moth. The density of codling moth eggs in most commercial orchards is very low and a large number of traps may be necessary to detect oviposition (Zoller and Zoller 2001). Females typically deposit eggs near or on fruit clusters that emit E,E-αF and other volatiles that stimulate oviposition (Sutherland *et al.* 1974). It is unclear whether adding a Et-*E*,*Z*-DD lure could further increase this stimulation. For example, the addition of Et-*E*,*Z*-DD to an attractive blend of E,E-αF and (*E*)-*β*-farnesene did enhance male upwind flight but not 'landing at source' in flight tunnel studies (Coracini *et al.* 2004). Studies addressing the influence of these volatile blends on short-range female orientation and oviposition have not been conducted. The cut-pear method is the only study that has stimulated codling moth oviposition in the field with the addition of kairomones (Zoller 2001), but the blend of volatiles and their emission rates from artificially injured pears has not been characterized. While a wide range of Et-*E*,*Z*-DD lure loadings was evaluated in our studies we have not compared the emission rate of Et-*E*,*Z*-DD from cut pears versus these synthetic lures. Very low Et-*E*,*Z*-DD lure loadings (1.0 – 100.0 µg) stimulated oviposition under laboratory conditions. However, higher rates of Et-*E*,*Z*-DD ($\geq$ 1.0 mg) are needed to attract adequate numbers of female moths to traps under field conditions (Light *et al.* 2001). The successful development of an egg trap for codling moth must consider the trade-off between increasing the number of eggs laid on or near the trap (short-range stimulation) versus increasing the likelihood of detecting a single egg near or on the trap (long-range attraction). The complexity of visual and olfactory cues that may stimulate short-range host location behaviors in codling moth is not well understood.

Additional factors associated with developing a practical egg-monitoring program for codling moth should be considered. Lombarkia and Derridj (2002) found that several primary water-soluble metabolites (sugar alcohols and sugars) isolated from apple leaves and fruits stimulated oviposition by codling moth. Whether the suitability of a plastic surface might be improved or the leaf and fruit surfaces enhanced by the addition of these or other chemicals should be investigated. Microencapsulated formulations of Et-*E*,*Z*-DD alone and in blends with other attractive volatiles could be applied to fruit clusters to create attractive point sources. Other physical aspects of the trap could be improved to increase oviposition, such as providing grooves or folds in the trap's surface and the influence of trap color (Knight and Miliczky 2003).

The attraction of codling moth adults and neonates plus the stimulation of ovi-

position by Et-*E*,*Z*-DD could be utilized to improve management of this pest (Light *et al.* 2001; Knight and Light 2001). Laboratory studies with Et-*E*,*Z*-DD have examined the use of a paste formulation laced with insecticides applied as coarse droplets to control larvae (A. L. K., unpublished data). The use of attractive kill stations for females has been evaluated in field trials and offers promise (Knight *et al.* 2002). Hughes *et al.* (2003) evaluated the use of apple odor and *E,E*-αF to disrupt host location of neonates and mated females in laboratory trials. They suggest that natural mortality of neonates could be increased with a competitive kairomone-based approach. A similar design using Et-*E*,*Z*-DD could be effective and should be further evaluated.

Another potential use of Et-*E*,*Z*-DD can be to increase oviposition by post-diapause field-collected strains of codling moth. The overwintering generation of field-collected populations of codling moth typically has a much lower fecundity and mating success under laboratory conditions than either laboratory-adapted or summer generation field-collected strains (Howell 1991). Increasing egg production of codling moth under laboratory conditions has been facilitated by the addition of ripe apple fruits, water, or molasses baits (Van Leeuwen 1947; Wearing *et al.* 1973). The use of a dry, long-lasting Et-*E*,*Z*-DD lure to increase egg laying would facilitate insecticide-susceptibility testing of field populations and in establishing laboratory colonies (Knight *et al.* 2001).

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Use of Ethyl (*E*,*Z*)-2,4-decadienoate in Codling Moth Management: Kairomone Species Specificity

A.L. KNIGHT[1] and D.M. LIGHT[2]

ABSTRACT

Ethyl (*E*,*Z*)-2,4-decadienoate (pear ester) is a kairomonal attractant for both male and female codling moth, *Cydia pomonella* (L.), in apple, pear and walnut. Studies were conducted in the western Untied States to evaluate the potential attractiveness of this kairomone for eight lepidopteran pests of these three crops, as well as, in cherry, peach/nectarine, apricot, plum, almond, pistachio, grape, kiwi, and citrus. The pear ester was loaded (10.0 mg) into gray halobutyl septa and insects were monitored with diamond- or delta-shaped sticky traps. Lures were not attractive to peach twig borer, *Anarsia lineatella* (Zeller); oriental fruit moth, *Cydia molesta* (Busck); omnivorous leafroller, *Platynota stultana* Walshingham; navel orangeworm, *Amyelois transitella* (Walker); apple fruitworm, *Lacanobia subjuncta* (Grote & Robinson); pandemis leafroller, *Pandemis pyrusana* (Kearfott); obliquebanded leafroller, *Choristoneura rosaceana* (Harris); and western tentiform leafminer, *Phyllonorycter mespiella* (Hüebner). Additional studies with *C. molesta* populations attacking apple and pear would be useful.

Key Words: *Cydia pomonella*, *Cydia molesta*, pear ester, host plant volatiles, monitoring

INTRODUCTION

Codling moth, *Cydia pomonella* (L.), is the key pest of pears, apples, and walnuts worldwide (Barnes 1991). Identification of the pear ester, ethyl (*E*,*Z*)-2,4-decadienoate, as a kairomone attractant for adult and larval stages of codling moth has allowed the development of several new approaches to successfully monitor and manage this pest (Light *et al.* 2001, Knight and Light 2001; Knight *et al.* 2002; Knight *et al.* 2005). Pear ester is a characteristic volatile of ripe pear (Jennings *et al.* 1964) and has not been detected in headspace volatiles of unripe pear fruit and is not known to be present in pear leaves (Shiota 1990, Miller *et al.* 1989) or in walnut fruit or leaves (Buttery *et al.* 2000). However, it has been detected as a minor constituent in ripe 'Red Delicious' apple (Berger *et al.* 1984) and in quince (Schimizu and Yoshihara 1977).

The attractiveness of pear ester for insects other than codling moth has been reported. The yellowjacket wasp *Vespula vidua* (Saussure) was caught in low numbers in traps baited with pear ester (Day and Jeanne 2001); and higher lure loadings (> 40.0 mg) in gray halobutyl elastomer septa are attractive to the western yellow jacket, *Vespula pennsylvanic* (Saussure) in apple (ALK, unpublished data). Low numbers of adult stink bugs, *Euschistus conspersus* Uhler, have occasionally been observed in or near traps baited with > 20.0 mg pear ester septa (ALK and DML, unpublished data).

The attractiveness of pear ester to other lepidopteran species has also been reported. Two polyphagous tortricid species, *Hedya nubiferana* Haworth and *Cydia fa-*

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giglandana (Zeller), were caught in traps baited with pear ester in Swedish apple orchards (Coracini *et al.* 2004) and in a mixed apple and cherry orchard in Italy (Schmidt *et al.* 2004). In addition, the pest species, *Cydia splendana* (Hübner) was caught in traps baited with pear ester in chestnut orchards (Schmidt *et al.* 2004).

The attractiveness of pear ester to lepidopteran pests sympatric with codling moth in pome fruits and walnuts in western North America has not been studied. This complex of pest species includes tortricid leafrollers, noctuid fruitworms and cutworms, and gracillarid leafminers (Beers *et al.* 1993; VanBuskirk *et al.* 1999). Six tortricid leafroller species feed on the developing buds, leaves, and external surface of apple and pear fruit: *Choristoneura rosaceana* (Harris), *Pandemis pyrusana* (Kearfott), *Platynota stultana* Walsingham, *Archips rosanus* (L.), *Argyrotaenia citrana* (Fernald), and *Archips argyrospilus* (Walker). The western tentiform leafminer, *Phyllonorycter mespiella* (Hüebner), is a common indirect gracillarid feeding beneath the epidermis of apple and pear leaves but causing little economic damage to the crop. Several cutworm and fruitworm noctuid species including *Xestia c-nigrum* (L.) and *Lacanobia subjuncta* (Grote & Robinson), are occasional pests of apple and pear orchards and feed on buds, leaves, and fruit (Barnett *et al.* 1991; Landolt 1998). The oriental fruit moth, *Cydia molesta* (Busck), is a key pest of stone fruits attacking both developing shoots and fruits (Rothschild and Vickers 1991). However, this species has expanded its host range recently and has become a significant pest of apple and pear in some geographical regions (Civolani *et al.* 1998; Usmani and Shearer 2001; Il'ichev *et al.* 2003).

Many of these lepidopteran pest species of pome fruit have a broad host range that can include cherry, peach/nectarine, grape, citrus, kiwi, and pistachio, as well as uncultivated hosts (Barnett *et al.* 1991; Beers *et al.* 1993). Host races of codling moth are reported to sporadically attack other crops such as plum, apricot, and almonds (Barnes 1991) and have been reported to attack cherry (Mote 1926). Within these crops other important lepidopteran pests can occur. The navel orangeworm, *Amyelois transitella* (Walker), attacks walnuts, pistachios, and almonds (Barnett *et al.* 1991). The peach twig borer, *Anarsia lineatella* (Zeller), is another key pest damaging shoots and fruits of almonds and stone fruits (Barnes *et al.* 1993).

The objective of this study was to evaluate the attractiveness of pear ester for eight important lepidopteran pests that are sympatric with codling moth among several host crops in Washington and California. In addition, the attractiveness of pear ester for *P. stultana*, *C. molesta*, and *A. lineatella* was evaluated across a range of crops that are not hosts for codling moth.

MATERIALS AND METHODS

Specificity Studies in Washington. Studies were conducted in apple (n = 15), pear (n = 10), cherry (n = 5), and peach (n = 10) orchards during 1999 to evaluate the species specificity of pear ester. Groups of three diamond-shaped sticky traps (Pherocon IIB, Trécé Inc., Adair, OK) baited with gray halobutyl elastomer septa (No. 1888, size No. 1, West Co., Phoenixville, PA) loaded with either 10.0 mg pear ester (93.7% A.I. purity, Aldrich Chemical, Minneapolis, MN), sex pheromone (proprietary loading, Trécé Inc., Adair, OK), or a hexane solvent were spaced more than 50 m apart and hung in the upper third of the canopy within each orchard. Studies were conducted in apple, pear, cherry and peach orchards located near Moxee, Wapato, and Brewster WA. *Lacanobia subjuncta* and *P. mespiella* were each monitored simultaneously in 10 pear orchards from 9 – 23 August and in 10 apple orchards from 16 – 30 August. Traps baited with the sex pheromone of *P.*

mespiella were not included in the pear study. *Pandemis pyrusana* was monitored in five apple and cherry blocks from 24 – 31 August. *Choristoneura rosaceana* was monitored in five apple blocks from 9 – 16 September. *Cydia molesta* and *A. lineatella* were monitored simultaneously in 10 peach orchards for 2 – 7 nights from 11 August to 7 September. Nontarget insects caught in traps were counted and broadly categorized by order (e.g. small dipterans, dermapterans) or family or super family (e.g. chrysopids, coccinellids, muscoid flies). Numbers of adult white apple leafhopper, *Typhlocyba pomaria* McAtee, and codling moth were recorded for all traps in apple and pear and for all crops, respectively.

Specificity studies in California. Studies were conducted from 18 August – 16 September 1999 in orchard blocks of mixed cultivars of peach, apricot, plum, almond, pistachio, grape, kiwi, and citrus at the University of California campus in Davis, CA; and at its germplasm repository research station at Wolfskill in Winters, CA. All crops except citrus were monitored for *P. stultana*. *Cydia molesta* and *A. lineatella* were monitored in all crops except grape, kiwi and citrus. *Amyelois transitella* was present in the almond blocks but was not specifically monitored due to the ineffectiveness of the sex pheromone-baited trap. Orchard blocks were monitored with 10.0 mg pear ester, species' sex pheromone, and a trap baited with a solvent blank lure placed in either wing-shaped or diamond-shaped sticky traps (Pherocon 1CP and IIB, Trécé Inc., Adair, OK). Sex pheromone lures are commercially available and were provided by Trécé Inc. Traps were typically placed in the mid canopy of orchards of each crop in a randomized block design along 2 – 4 replicate orchard rows separated by 50 – 80 m.

Data analysis. Analysis of variance (ANOVA) was used to detect significant differences in mean moth catch per trap per night among the sex pheromone, pear ester, and solvent lures for each species, $P < 0.05$ (Analytical Software 2000). Means in significant ANOVA's were separated with a least significant difference test. A paired t-test was used to compare the catch of selected nontarget insects in traps baited with pear ester or blank septa.

RESULTS

Specificity studies in Washington. Species-specific sex pheromone-baited traps caught male *P. pyrusana*, *C. rosaceana*, *L. subjuncta*, *P. mespiella*, *A. lineatella*, and *C. molesta* across apple, pear, cherry, and peach orchards (Table 1). Mean daily moth catches in these sex pheromone-baited traps were significantly higher than moth catches in traps baited with pear ester or with blank lures. No differences occurred in the catch of each of these species in any crop between traps baited with pear ester and blank lure-baited traps. Low numbers of codling moth were caught per day in traps baited with pear ester in apple (0.24 ± 0.04) (mean $\pm$ SEM), pear (0.10 ± 0.06), cherry (0.03 ± 0.03), and peach (0.17 ± 0.13), indicating the pear ester lures were active.

Various other insect species were caught in sticky traps baited with sex pheromones, pear ester, or a blank lure: including low sporadic numbers of earwigs, lacewings, ladybird beetles, microhymenopterans, and various species of bees. Small dipteran species were commonly caught in traps though generally in low numbers. The two most common nontargets in apple and pear blocks in Washington during these trials were muscoid flies (means of 4 – 5 flies per trap) and white apple leafhopper, *T. pomaria* (means of 12 – 13 adults per trap). However, no significant difference in their densities were found in traps baited with either pear ester or blank lures for either group, P's = 0.48 and 0.61, respectively (paired t-tests).

Table 1.
Captures of moths in traps baited with either a conspecific sex pheromone, ethyl (2*E*,4*Z*)-2,4-decadienoate (pear ester), or a blank lure in fruit orchards in Washington State.[1]

Pest species	Crop (no. orchards)	Mean ± SEM moths/trap/night			ANOVA: *F*-value; df; *P*-value
		Conspecific sex pheromone	Pear ester	Blank	
Pandemis pyrusana	Apple (5)	7.6 ± 0.7a	0.00 ± 0.00b	0.08 ± 0.06b	132.0; 2, 12; < 0.001
Pandemis pyrusana	Cherry (5)	5.4 ± 1.8a	0.00 ± 0.00b	0.02 ± 0.02b	8.43; 2, 12; < 0.01
Choristoneura rosaceana	Apple (5)	4.0 ± 0.8a	0.00 ± 0.00b	0.00 ± 0.00b	25.8; 2, 12; < 0.001
Lacanobia subjuncta	Apple (10)	0.8 ± 0.1a	0.00 ± 0.00b	0.03 ± 0.02b	122.0; 2, 27; < 0.001
Lacanobia subjuncta	Pear (10)	1.0 ± 0.1a	0.00 ± 0.00b	0.02 ± 0.02b	53.7; 2, 27; < 0.001
Phyllonorycter mespiella	Apple (10)	553.6 ± 19.2a	3.7 ± 1.3b	3.9 ± 1.4b	814.0; 2, 27; < 0.001
Phyllonorycter mespiella	Pear (10)	-	0.5 ± 0.3	0.4 ± 0.1	0.14; 1, 18; = 0.71
Anarsia lineatella	Peach (10)	13.8 ± 4.0a	0.0 ± 0.0b	0.1 ± 0.1b	11.90; 2, 27; < 0.001
Cydia molesta	Peach (10)	1.4 ± 0.7a	0.0 ± 0.0b	0.0 ± 0.0b	4.69; 2, 27; < 0.05

[1] Row means followed by a different letter were significantly different, $P < 0.05$ LSD test.

Specificity studies in California. *A. lineatella, C. molesta*, and *P. stultana* males were caught in sex pheromone-baited traps in peach, apricot, plum, almond and pistachio orchards in California (Table 2). In addition, male *P. stultana* were trapped in grape and kiwi sites. No moth species were caught in pear ester- or solvent-baited traps in any crop other than low catches of codling moth in blocks of peaches, almonds, and citrus more than 100 m from pome fruit orchards. These moth counts in the sex pheromone-baited traps were all significantly different than the zero catch in the pear ester-baited traps (P's < 0.01).

DISCUSSION

The pear ester is a strong attractant for codling moth adults and has improved monitoring of this pest in walnut (Light *et al.* 2001), apple (Thwaite *et al.* 2004), and pear (Knight *et al.* 2005). It also has demonstrated potential to improve control via lure and kill approaches (Knight *et al.* 2002) and disruption of oviposition

Table 2.
Captures of moths in traps baited with either a conspecific sex pheromone, ethyl (2*E*,4*Z*)-2,4-decadienoate (pear ester), or a blank lure in fruit orchards and vineyards in California.

	Mean ± SEM moths/trap/night					
	Conspecific sex pheromone[1]			Pear ester		Blank
Crop	*Anarsia lineatella*	*Cydia molesta*	*Platynota stultana*	All other moth species	*Cydia pomonella*	Solvent Control
Peach	14.01 ± 2.55	14.89 ± 1.70	1.25 ± 0.33	0	0.07 ± 0.04	0
Apricot	10.68 ± 1.83	0.32 ± 0.12	2.46 ± 0.77	0	0	0
Plum	5.93 ± 2.44	0.32 ± 0.12	5.50 ± 1.02	0	0	0
Almond	7.86 ± 2.38	3.75 ± 0.53	0.79 ± 0.33	0	0.18 ± 0.14	0
Pistachio	3.04 ± 1.65	0.39 ± 0.15	0.57 ± 0.29	0	0	0
Grape	-	-	3.64 ± 0.95	0	0	0
Kiwi	-	-	11.38 ± 1.83	0	0	0
Citrus	-	-	-	0	0.04 ± 0.04	0

[1] Mean moth catch in sex pheromone-baited traps were all significantly different than moth catch in pear ester-baited traps, $P < 0.01$ (ANOVA).

(Pasqualini *et al.* 2004). Conversely, our studies reported here have demonstrated that pear ester is not attractive for eight lepidopteran pest species of a number of important horticultural crops in California and Washington. The majority of these lepidopteran pests either attack crops that do not produce pear ester or feed and oviposit primarily on foliage of pear or apple that also lack pear ester. Species that are known to be attractive to pear ester either feed on ripe pear such as yellowjackets (Akre and Davis 1979) and stink bugs (Beers *et al.* 1993); share the major sex pheromone component, (*E,E*)-8,10-dodecadien-1-ol with codling moth, such as *C. fagiglandana* and *H. nubiferana*, or can detect this compound, such as *C. splendana* (Schmidt *et al.* 2004), or have a closely related sex pheromone, such as methyl (*E,Z*)-2,4-decadieonoate for *Euschistus* spp. stink bugs (Aldrich *et al.* 1991).

Among the various lepidopteran pests in our study only *C. molesta* is a true internal fruit feeder that also attacks pear. Both codling moth and *C. molesta* can also attack quince (Cravedi and Ughini 1992), another fruit that can release pear ester (Schimizu and Yoshihara 1977). Pear host races of *C. molesta* have been reported in Australia (Il'ichev *et al.* 2003) and Italy (Civolani *et al.* 1998); however, the attractiveness of pear ester for *C. molesta* in these regions has not been examined. Efforts to improve monitoring of *C. molesta* populations, particularly in orchards treated with sex pheromone mating disruption, have focused on the identification of host plant volatiles attractive for females (Natale *et al.* 2003). While adult populations of *C. molesta* trapped in peach, apricot, plum, almond, and pistachios in our study were not attractive to pear ester, subsequent studies will evaluate the seasonal attractiveness of pear ester for *C. molesta* populations feeding on apple or pear.

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Managing Codling Moth (Lepidoptera: Tortricidae) with an Internal Grid of Either Aerosol Puffers or Dispenser Clusters Plus Border Applications of Individual Dispensers

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ABSTRACT

Field trials run from 2001 to 2003 evaluated the effectiveness of a combination of hand-applied sex pheromone dispensers (Isomate-C) applied on the perimeter of apple orchards with an internal grid of either pressurized aerosol emitters (puffers) or clusters of dispensers for control of codling moth, *Cydia pomonella* (L.). Puffers were placed in a grid at 1 per ha, while the dispenser clusters were applied at 4 - 8 per ha. Puffers were programmed to release either 240 or 360 mg (*E*, *E*)-8-10-dodecadienol (codlemone) per day in 48 puffs (every 15 min from 1500 – 0300 h). Dispensers were grouped in clusters of 50 (Isomate-C TT) or 100 (Isomate-C PLUS) releasing 56 and 33 mg codlemone per d, respectively. No significant differences were found in levels of fruit injury in puffer-treated orchards paired with similar orchards treated with 500 Isomate-C PLUS individually applied dispensers per ha. Similarly no significant differences in fruit injury were found in orchards treated with individually applied dispensers versus orchards treated with Isomate-C PLUS dispensers (100 per cluster) placed in screened cages or Isomate-C TT dispensers (50 per cluster) hung from plastic disks. Levels of fruit injury, however, were significantly higher in orchards treated with Isomate-C PLUS dispensers (100 per cluster) hung from plastic disks versus in orchards treated with individually applied dispensers. This later poor performance of the Isomate-C PLUS clusters was associated with its more restricted spacing of dispensers within the cluster and a significant reduction in the weight loss of dispensers compared with dispensers applied individually. These studies suggest that the use of puffers can effectively lower the cost of codling moth management through reductions in sex pheromone puff volume and emitter density.

Key Words: mating disruption, sex pheremones, puffers, apple

INTRODUCTION

Since 1990, uniformly distributed hand-applied dispensers loaded with (*E*, *E*)-8-10-dodecadienol (codlemone) have been the most commonly used approach to disrupt mating of codling moth, *Cydia pomonella* (L.) in North American tree fruit and nut crops (Thomson *et al.* 2000). Major problems associated with the use of hand-applied dispensers have been maintaining the chemical stability of codlemone (Brown *et al.* 1992; Millar 1995), seasonal variability in emission rates primarily due to changes in temperature (Howell 1992; Knight 1995a), material cost (Alway 1997), and the labor cost of applying hundreds of dispensers per hectare (Knight 1995b; Williamson *et al.* 1996). High emission, timer-activated mechanical aerosol dispensers (puffers) have been suggested as an alternative that can solve some of these problems (Mafra-Neto and Baker 1996; Shorey *et al.* 1996; Isaacs *et al.* 1999). Puffers are used at a low density, can protect sex pheromones from UV degradation and oxidation, allow the application of a consistent pheromone release rate

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throughout the season, and allow users to adjust the cycle and periodicity of sex pheromone release (Shorey *et al.* 1996).

Shorey and coworkers postulated that effective mating disruption depended on the concentration of sex pheromone released per area and was not significantly affected by the spacing between individual point sources (Farkas *et al.* 1974; Shorey *et al.* 1996; Shorey and Gerber 1996a, b, c). They showed that the distance between pheromone sources could be quite large. For example, a perimeter application of emitters 100 m apart effectively disrupted *Platynota stultana* (Walsingham) and *Spodoptera exigua* (Hübner) mating (Shorey *et al.* 1996).

Puffers were initially evaluated for codling moth in walnuts where tree height and large canopy size had precluded the adoption of hand-applied dispensers (Shorey and Gerber 1996b). A single orchard was treated with 2.3 puffers per ha with units spaced 40 m apart along its perimeter. Puffers were programmed to release approximately 5.0 mg codlemone per puff every 30 min (254 mg/ha per d). Moth catches in traps baited with synthetic lures and virgin females were reduced 95 and 98% in this orchard versus in traps placed in an untreated orchard (Shorey and Gerber 1996b). The current standard protocol for codling moth control with puffers recommends the use of 2.5 – 5.0 puffers per ha arranged primarily around the perimeter of orchards. However, in large blocks (> 16 ha) a few puffers are placed along the upwind interior of the orchards (Elkins 2002).

Four major problems have occurred with the use of puffers for management of codling moth. First, placement of puffers on the perimeter of orchards has not been effective in preventing injury along the upwind edges of the orchards (Shorey *et al.* 1998). Greater wind speed and turbulence plus higher moth population density along orchard borders are problematic with this approach (Milli *et al.* 1997). A second problem has been their high cost. The fixed cost of individual cabinets housing the electronics ($40 amortized over 5 y) plus the yearly cost of the disposable pheromone-loaded canisters ($80) often limits the number of units deployed. Hand-applied dispensers in Washington State are typically applied at rates of 500 per ha and cost $125 per ha (Alway 1997). To be cost competitive puffer density should be 1.5 – 2.0 units per ha. A third problem has been the loss of units during the season due to wind, vandalism, and a variety of mechanical malfunctions. The unreliable performance of puffers has required expensive maintenance and frequent monitoring of units (Knight 2002). A fourth problem has been the occurrence of severe marking of fruit and foliar phytotoxicity surrounding units due to incidental contact with the sex pheromone solution (Giroux and Miller 2001).

An alternative approach was developed that alleviates some of these constraints (Knight 2002). Orchards are treated with an internal grid of puffers spaced 50 m from the border and 100 m apart (one puffer per ha) in combination with a perimeter application of hand-applied dispensers (1,000 per ha). This design was tested in apple orchards for codling moth alone or codling moth and obliquebanded leafroller, *Choristoneura rosaceana* (Harris), using canisters loaded with the sex pheromone of both species (Knight 2002). This method provided good control of both pests at a lower cost per ha than the use of uniformly distributed hand applied dispensers. However, these studies were only conducted in large, regular-shaped orchards with flat terrain and have not sufficiently addressed the utility of this approach in smaller, irregular-shaped orchards with sloping topography (Knight 2002). The initial success of this approach also suggests that lower puffer volumes of sex pheromone should be evaluated. Unfortunately, problems with the reliability of the puffer units and phytotoxic effects persisted in this study and grower adoption has been slowed by these problems. An alternative design has been proposed that would replace the mechanical puffers with

clusters of hand-applied dispensers to generate high emission point sources (Knight 2002).

Herein, I report results from field evaluations conducted in apple from 2001-03 using grids of both puffers and clusters of dispensers to manage codling moth. Studies were conducted with puffers emitting two rates of sex pheromone and with four types of clusters that varied in dispenser density, dispenser type, and dispenser spacing within the cluster. Results demonstrate that an internal grid of widely spaced emitters can be effectively used as part of an integrated management approach for codling moth and can further reduce the costs associated with using sex pheromones.

MATERIALS AND METHODS

Studies were conducted in 20 – 60 apple orchards of several cultivars (primarily 'Delicious', 'Fuji' and 'Granny Smith') near Brewster, WA each year from 2001-03. All orchards evaluated in 2001 were 16 ha with level terrain. Orchards in 2002-03 varied in size from 4.0 – 16.0 ha and very often were irregular in shape with a moderately sloping terrain (3.0 – 6.0° slope).

Field trials were conducted to evaluate the effectiveness of a combination of hand-applied sex pheromone dispensers (Isomate-C PLUS, Pacific Biocontrol, Vancouver, WA) applied on the perimeter of apple orchards with an internal grid of either pressurized aerosol emitters (puffers) or clusters of hand-applied dispensers (Isomate C Plus and Isomate C TT, Pacific Biocontrol, Vancouver, WA). Puffer cabinets (Paramount Puffer®, Paramount Farming, Bakersville, CA) were spaced in an internal 100 x 100 m grid (1 per ha) beginning 50 m from the edges of the orchard. Orchards treated with clusters of dispensers received four clusters of dispensers per ha spaced in an internal 50 x 50 m grid, except for five orchards in 2003 that were treated with eight clusters of dispensers per ha spaced 35 x 35 m apart. Cluster grids were spaced beginning 25 m from the edge of the orchard. The perimeter of these orchards was also treated with a 10-20 m wide band of hand-applied Isomate-C PLUS dispensers at a rate of 1,000 dispensers per ha.

Puffers were evaluated in 2001 and 2002 in 22 orchards. The cabinet of puffers is constructed of high-density polyethylene plastic (32 x 15 x 12 cm) and powered by four 'AA' batteries. Puffer operation was controlled by a remote controller and puffers operated only at temperatures > 10 °C. Cabinets were mounted on wood blocks and attached with a metal clip to trees in the upper third of the canopy. Puffer canisters are pressurized metal cylinders loaded with either a 25.0% or a 16.7% solution of codlemone plus solvents and propellants. Canisters emit a 30 mg spray through a solenoid-metered valve every 15 min from 1500 – 0300 h. Studies were conducted in 2001 with puffers emitting either a 7.5 or 5.0 mg A.I. dose (codlemone). Studies in 2002 tested only the 5.0 mg dose.

The effectiveness of using clusters of Isomate-C dispensers was evaluated in 39 orchards from 2001-03. Two different polyethylene Isomate-C dispensers were used in clusters, Isomate-C PLUS and Isomate-C TT. Both dispensers are loaded with a 60:33:7 blend of (*E*, *E*)-8-10-dodecadien-1-ol, dodecanol, and tetradecanol. Isomate-C PLUS and Isomate-C TT dispensers were loaded with 182.3 and 382.4 mg active ingredients, respectively. Only Isomate-C PLUS dispensers were used in 2001 and they were clustered in square, screened boxes (19.0 x 19.0 x 21.0 cm). One hundred dispensers were placed vertically in the screened boxes in alternating cells and were spaced on average (SE) 2.4 (0.1) cm apart. The screened sides of the box had a 0.41 cm^2 mesh and the ends of the box were constructed with a 0.10 cm^2 screen to prevent dispensers from falling out. Plastic bucket lids (21.7 cm diameter) were used to hold clusters of dis-

pensers during 2002-03. The plastic lids had either 100 slits (0.15 x 0.7 cm) or 50 0.48 cm-diameter holes cut in the lids for Isomate-C PLUS or Isomate-C TT dispensers, respectively. One end of each dispenser was inserted vertically into the plastic lid. The mean (SE) spacing of Isomate-C PLUS and Isomate-C TT dispensers hanging from the lid was 1.3 (0.2) cm and 3.0 (0.2) cm, respectively. Studies were conducted with Isomate-C PLUS in 13 orchards in 2002 and with Isomate-C TT in 11 and 10 orchards in 2002 and 2003, respectively. During 2003 five orchards each were treated with four or eight clusters per ha. The screened boxes and plastic lids were attached to poles with wire hangers. Clusters of dispensers were placed in the upper third of the canopy.

Comparison orchards (controls) were selected each year and paired with orchards treated with puffers or dispenser clusters. Orchard pairs were selected based on similarity in size, proximity, cultivar, ownership, spray practices and pest pressure (based on percent fruit injury the previous year). All comparison orchards were treated with Isomate-C PLUS dispensers applied at a rate of 500 dispensers per ha.

Pheromone lure-baited delta-shaped traps were deployed in all orchards at a density of 1 per 2 ha placed around the perimeter of each orchard 10 m from the edge. Orchards treated with dispenser clusters in 2001 and 2003 plus the associated comparison orchards were also monitored with two (2001) or three (2003) pear ester-baited traps placed 50 m from the edge of orchards and >100 m from the nearest trap. Pear ester lures were used in these two studies to provide a second measure of moth population density that would be more independent of the sex pheromone treatment. Trécé Inc. (Adair, OK) provided all monitoring traps and lures. Traps were placed in the upper third of the orchards' canopy using a permanent PVC pole. Sex pheromone-baited traps were checked weekly and pear ester-baited traps were checked every 4 wk. Lures were replaced after 8 wk and removable sticky trap liners were replaced as needed.

Pre-harvest fruit injury was assessed by sampling 30 fruit from 20 trees selected within each quadrant of each orchard (2,400 fruit sampled per orchard). An equal number of fruit were sampled from the interior and from the edge (< 30 m from the perimeter) of each quadrant. Spray records were obtained from the growers and field managers at the end of each season.

The weight loss of each dispenser type for dispensers applied individually or within a cluster was analyzed in 2002. Six new dispensers of each type were weighed on 29 April. Isomate-C Plus dispensers were then twisted on to a plastic clip and attached to branches in a pear orchard. Isomate-C TT dispensers were placed over branches in the same orchard. Five new dispensers were randomly selected from the outer rim and five from the center of four clusters and were weighed. Clusters were hung individually in pear trees spaced 10 m apart. All dispensers were reweighed on 25 September.

Statistical analysis. Mean moth catch per trap, number of insecticide sprays applied for codling moth, and percent fruit injury in orchards treated with uniformly distributed individual dispensers versus grids of puffers or dispenser clusters were analyzed with the Wilcoxon Rank Sum test (Analytical Software 2000). The mean daily weight loss for dispensers applied individually or placed in the middle or outer rim of clusters in 2002 was analyzed for each dispenser type with a Kruskal-Wallis test (Analytical Software 2000).

RESULTS

No significant differences were found for mean moth catch, number of insecticide sprays applied, and codling moth fruit injury between puffer-treated and individually applied dispenser-treated orchards at either puffer emission rate (Table 1). Ap-

Table 1.
Summary data for paired apple orchards treated with either an internal grid of one aerosol puffer per ha plus a perimeter treatment of Isomate-C PLUS dispensers or 500 Isomate-C PLUS dispensers per ha. Puffers released either 5.0 or 7.5 mg sex pheromone per puff. No significant differences were found in moth catch, number of insecticide sprays applied, and percent fruit injury between the paired puffer and Isomate-treated orchards, Wilcoxin Signed Rank tests, two-tailed *P*-value > 0.05.

Puff-size (mg)	Year (no. orchards)	Mean (SE) moth catch per trap		Mean (SE) no. sprays		Mean (SE) % fruit injury	
		Puffers	Dispensers	Puffers	Dispensers	Puffers	Dispensers
7.5	2001 (10)	1.5 (0.8)	2.5 (1.1)	0.8 (0.2)	0.9 (0.2)	0.22 (0.15)	0.20 (0.12)
5.0	2001 (5)	4.3 (1.8)	5.7 (2.1)	1.3 (0.4)	2.4 (0.6)	0.20 (0.13)	0.54 (0.20)
5.0	2002 (7)	6.9 (1.9)	7.8 (3.6)	1.5 (0.4)	1.4 (0.4)	0.12 (0.03)	0.08 (0.07)
5.0	All (12)	5.8 (1.5)	6.9 (3.0)	1.4 (0.2)	1.8 (0.3)	0.15 (0.06)	0.27 (0.11)

ple orchards had low codling moth population densities as evidenced by low cumulative moth catches in sex pheromone-baited traps (< 10 moths per season) (Table 1). Growers supplemented their use of puffers and dispensers with 1 – 3 insecticide sprays per season. Insecticides applied for codling moth included azinphosmethyl, phosmet, and methoxyfenozide. Nearly half of all sprays were applied only to the borders of orchards.

Orchards treated with clusters of dispensers had similar moth catches and supplemental insecticide sprays applied as orchards treated with individual dispensers during all three years (Table 2). Codling moth fruit injury in orchards treated with individually applied dispensers paired with orchards treated with either screened box clusters with Isomate-C Plus or plastic lid clusters of Isomate-C TT dispensers were not significantly different in any of the three years (Table 2). However, fruit injury was significantly higher during 2002 in orchards treated with plastic lid clusters of Isomate-C Plus dispensers than in the paired orchards treated with individually applied dispensers (Table 2). During 2003, orchards treated with 4.0 and 8.0 clusters per ha loaded with Isomate-C TT dispensers had similar levels of codling moth injury as orchards treated with 500 or 1,000 dispensers per ha, respectively (Table 2)

The mean weight loss of dispensers within plastic lid clusters versus those individually applied varied between dispenser type (Table 3). No difference was found in the mean weight loss of Isomate-C TT dispensers placed in clusters versus dispensers applied individually. The mean daily emission rate of codlemone from these clusters during the season averaged 55.6 mg. In comparison, Isomate-C Plus dispensers in both the center and edge of clusters loss significantly less weight during the season than dispensers applied individually (Table 3). The mean daily emission rate of codlemone from these clusters averaged only 32.7 mg during the season.

DISCUSSION

These studies show that treating orchards with a high-density application of dispensers on the perimeter and a widely spaced grid of high emission emitters internally can be substituted for the application of hundreds of individually applied dispensers to manage codling moth in apple. The use of either pressurized canisters releasing puffs of sex pheromone every 15 min or the continuous passive diffusion of

Table 2.
Summary data for paired apple orchards treated with either an internal grid of clusters of Isomate-C dispensers at 4.0 or 8.0 clusters per ha plus a perimeter treatment of Isomate-C PLUS dispensers or treated with 500 – 1,000 Isomate-C PLUS dispensers per ha. Clusters were baited with either 100 Isomate-C PLUS or 50 Isomate-C TT dispensers.[1]

Treatment per ha[2] (# paired orchards)	Mean (SE) moth catch per trap		Mean (SE) no. cover sprays	Mean (SE) % fruit injury
	Sex pheromone	Kairomone		
2001				
4 C-Plus box clusters (5)	2.6 (0.9)	3.0 (1.0)	0.8 (0.2)	0.00 (0.00)
500 C-Plus dispensers	2.8 (0.8)	3.4 (0.7)	1.0 (0.0)	0.12 (0.06)
2002				
4 C-TT lid clusters (11)	8.4 (2.7)	-	1.5 (0.5)	0.15 (0.08)
500 C-Plus dispensers	10.1 (3.6)	-	2.0 (0.5)	0.14 (0.06)
4 C-Plus lid clusters (13)	10.7 (2.9)	-	1.6 (0.4)	0.14 (0.06)**
500 C-Plus dispensers	6.5 (2.1)	-	1.8 (0.4)	0.05 (0.03)**
2003				
8 C-TT lid clusters (5)	2.4 (1.5)	8.8 (6.1)	1.8 (0.4)	0.20 (0.20)
1,000 C-Plus dispensers	1.2 (0.6)	2.2 (0.6)	1.8 (0.4)	0.00 (0.00)
4 C-TT lid clusters (5)	2.6 (1.6)	4.8 (1.9)	1.4 (0.6)	0.13 (0.10)
500 C-Plus dispensers	7.4 (5.9)	3.8 (2.6)	1.8 (0.4)	0.00 (0.00)

[1] '**' P-value < 0.01, two-tailed Wilcoxin Signed Rank test. All other statistical comparisons were not significant, $P > 0.05$.
[2] One hundred Isomate C-PLUS dispensers were placed in screened boxes in 2001 and attached to plastic lids in 2002. Fifty Isomate C-TT dispensers were attached to plastic lids in 2002-03.

Table 3.
Mean (SE) weight loss (mg per d) of dispensers aged in the field, 29 April - 25 September 2002[1]

Dispenser	Individual dispenser	Dispenser in the center of cluster	Dispenser on the outer rim of cluster	P-value
Isomate-C TT	1.74 (0.11)	1.84 (0.04)	1.87 (0.05)	*0.52*
Isomate-C PLUS	0.71 (0.08)a	0.56 (0.02)b	0.53 (0.02)b	*< 0.01*

[1] Row means followed by a different letter were significantly different, $P < 0.05$, Kruskal-Wallis one-way nonparametric analysis of variance using a chi-squared approximation.

sex pheromone from clusters of dispensers were both effective. Charmillot *et al.* (1995) used a perimeter-only application of sex pheromone dispensers to reduce injury from *Lobesia botrana* Denis & Schiffinmüller in vineyards, and they were the first to suggest a grid design. A similar approach for codling moth where dispensers are applied only to the perimeter of orchards has not been tested.

Most of the orchards treated with sex pheromones in these studies were also sprayed with insecticides for control of codling moth. Thus it is not possible to

assess the effectiveness of the sex pheromone treatments alone. In general, sex pheromone is considered to be an important part of an integrated management program for codling moth, and can rarely be used as a single control tactic (Brunner *et al.* 2002). The latest survey of insecticide usage in Washington State reported that on average three to four sprays are applied for codling moth (National Agricultural Statistical Service 2002). Many orchards in the Brewster area have been treated with three to six insecticide applications in the past few years due to increased pest populations (A.L.K., unpublished data). Based on these data the use of sex pheromone in our study reduced insecticide use 40-60% compared with conventionally treated orchards.

Nearly half of all insecticides were applied only to the borders of orchards in our study. Codling moth injury most commonly occurs on the borders of orchards regardless of whether orchards are treated with hand-applied dispensers (Pfeiffer *et al.* 1993), treated with puffers placed on the perimeter (Elkins 2002), or treated only with insecticides (Madsen *et al.* 1975). Effective disruption of codling moth along border areas will remain problematic due to this area's reduced canopy structure and greater wind speed and turbulence that can reduce the sex pheromone concentration (Milli *et al.* 1997). Trimble and Vickers (2000) found that codling moth could be effectively managed over a three-year period in Ontario apple orchards with only border insecticide applications. The integration of border sprays with an internal grid of puffers/clusters may be an effective management alternative that has not yet been evaluated.

The internal grid design has several operational advantages over the standard hand application of hundreds of dispensers per hectare including cost savings and ease of use. Grouping the same number of dispensers into clusters reduced application costs up to 35% among growers that I surveyed. Yet, this savings for growers was minimal because application costs for hand-applied dispensers are low, $15 for 500 dispensers per ha (Knight 1995b). The application and servicing of puffers was not measured in this study but was reported earlier to be only $3 per puffer (Elkins 2002). The material cost of the puffer grid design was slightly less expensive than the use of reduced rates of hand-applied dispensers ($111 versus $125 per ha), except for the high fixed cost of the remote control ($350).

Further reductions in the cost of an effective management program using clusters of dispensers are unlikely. Clusters loaded with 100 Isomate-C Plus dispensers did not perform as well as the standard program and were estimated to release 30 mg codlemone per d. Clusters with 50 Isomate-C TT dispensers released 40% more codlemone per d than clusters of 100 Isomate-C PLUS and provided effective control. Further evaluation of clusters loaded with 30 – 50 Isomate-C TT dispensers might establish a minimum threshold needed with this design. However, the complex interplay of factors impacting the population dynamics of codling moth in commercial pheromone-treated orchards would likely obscure any marginal improvement in performance. Instead, additional cost savings can more likely be achieved by reducing the amount of sex pheromone loaded in puffers.

The amount of sex pheromone loaded in Paramount Puffers® used in Washington State orchards could be reduced 50% by lowering the pheromone puff from 7.5 to 5.0 mg and the longevity of the pheromone loading in the canister from 200 to 150 d. Unlike in California, the flight period of codling moth lasts approximately 150 d from late April to mid September in Washington State. Further refinements in the arrangement of puffers especially when supplemented with insecticides could further reduce the cost. Studies have shown that individual puffers can disrupt male codling moth orientation to traps placed 200 – 450 m downwind (Shorey and Gerber 1996b, Cave *et al.* 2001), and suggest that their density could be reduced to one

per several hectares. Puffer densities could likely be reduced in large, contiguous plantings. For example, the density of puffers declined from 4.0 to 2.8 per ha as the size of an area wide pear project in Lake County, California increased from 100 to 531 ha over a five-year period (Elkins 2002).

Puffers have been demonstrated to be an effective tool to manage codling moth under a variety of deployments (Elkins 2002, Knight 2002). The use of dispenser clusters was developed as an alternative due to the unreliability of puffers (Knight 2002). Yet, the characteristics of dispensers currently limit the maximum emission rate of codlemone from clusters. Thus, increasing the emission rates from clusters or the density of clusters will also increase the cost of this approach. Puffers allow greater flexibility in adjusting the emission rate and timing of release. Improvements in the operation and reliability of puffers will enhance their adoption. The use of an internal grid design may allow the development of a lower cost, effective management program for codling moth to be developed.

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Improved Deposition and Performance of a Microencapsulated Sex Pheromone Formulation for Codling Moth (Lepidoptera: Tortricidae) with a Low Volume Application

A.L. KNIGHT[1] and T.E. LARSEN[2]

ABSTRACT

Experiments were conducted to evaluate the deposition pattern and effectiveness in disrupting male orientation to virgin female-baited traps of a microencapsulated sex pheromone formulation for codling moth, *Cydia pomonella* (L.) in apple, *Malus domestica* Bordhausen. The efficacy of two application techniques was evaluated in field trials with the microencapsulated formulation Checkmate® CM-F: a high volume (926 liters per ha) application with an air blast sprayer and a low volume (46 liters per ha) application with a custom-built vertical boom sprayer. These treatments were compared to an unsprayed control and a control treatment where the formulation was applied directly on the ground within the plots. Disruption of virgin female-baited traps was significantly greater in the low volume versus the air blast application and versus the two types of control plots. Levels of disruption in the air blast-sprayed plots were not different from untreated plots or in plots where the sprayable sex pheromone was applied directly on the ground. A significant increase in the proportion of traps catching moths occurred in week 4. A significant interaction occurred among the effects of spray method, tree canopy position, and leaf surface on microcapsule deposition. This interaction was likely due to the low rate of deposition of microcapsules on the undersides of leaves in the lower canopy with the low volume sprayer. The low volume sprayer deposited significantly more microcapsules in the upper canopy than the air blast sprayer. Significantly more microcapsules were deposited on the underside versus the top of leaves in the upper canopy with the air blast but not with the low volume sprayer.

Key Words: sex pheromone, mating disruption, apple, pest management

INTRODUCTION

Various techniques have been developed to achieve mating disruption of codling moth, *Cydia pomonella* (L.), by treating orchards with controlled release devices (Vickers and Rothschild 1991). The use of sex pheromones in the western U.S. has been adopted rapidly since 1991 and is used on nearly 40,000 ha in Washington State alone (Brunner *et al.* 2002). Various hand-applied dispensers registered for codling moth have accounted for > 90% of this treated acreage (Thomson *et al.* 2000). Concurrently, microencapsulated sprayable formulations have been tested for codling moth (Knight 2000), but have not yet been widely adopted.

The ease of applying microcapsules with conventional equipment is a major factor generating grower's interest in sprayables (Campion 1976, Doane 1999). Sprayable sex pheromone formulations allow pest managers to treat crops with tall canopies, such as walnuts, and they increase grower's flexibility in adjusting application rates and timings during the season. In addition, microcapsules can be tank-mixed with other pesticides and can easily be included within a grower's inte-

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grated control program.

Conversely, a major limitation affecting the adoption of sprayable pheromone formulations has been their relatively short residual activity (Färbert *et al.* 1997). The emission profile from microcapsules typically exhibits a large initial burst followed by a sharp decline (Hall and Marrs 1989). Microcapsules for codling moth have been applied every four weeks during the growing season, but significant disruption of moth catches may occur for only one to two weeks (Knight 2000). Any efforts to extend the current activity of microcapsules will speed the adoption of this technology for codling moth management.

A large number of factors are likely involved in the successful use of microcapsules, including their emission profile over time, their structural integrity, the chemical stability of the sex pheromone, and density initially deposited and retained over time in the active area of the crop.

Surprisingly little is known about how these factors affect the performance of sprayable formulations for codling moth. The addition of antioxidant and UV stabilizers has reportedly improved the chemical stability of codlemone within a sprayable formulation (Eng *et al.* 2003). Laboratory studies have shown that differences in the epicuticular wax layer and the degree of pubescence of various plant tissues can affect microcapsule deposition rates in apple (Waldstein and Gut 2003, Knight *et al.* 2004). In addition, water has been shown to be a major factor causing dislodgement of microcapsules from leaves (Knight *et al.* 2004). Other factors, such as wind, abrasion, or other environmental factors impacting microcapsule retention under field conditions have not been investigated.

Adjusting one or more application parameters to increase either the deposition and/or retention of microcapsules over time may be a useful approach to extend the efficacy of sprayables for codling moth. Increasing the rate of application would likely also increase the density of microcapsules deposited in the canopy, but this approach is restricted by the comparable costs of alternative pest management tactics. Applying the same total rate of sex pheromone per season using more frequent applications of low rates of sprayables has been an effective compromise for some pest / crop systems (Polavarapu *et al.* 2001), but have not been successful in limited tests with codling moth (Hull *et al.* 2004). An additional parameter that may improve the performance of sprayables over time could be the refinement of the application technique.

Many pesticides including sprayables are applied in tree crops as concentrated sprays in spray volumes of 500-1,000 liters/ha using high velocity, air blast sprayers (Barnett *et al.* 1991). The use of these concentrated sprays can reduce application costs and avoid excessive leaf run-off as well as spray drift out of the orchard. Various factors on the sprayer are adjusted to generate a uniform spray coverage within the canopy, including tractor speed, spray velocity, and nozzle number, orifice size, and orientation (Byers *et al.* 1984). Pesticides are typically not applied with spray volumes <500 liters/ha in pome fruit orchards due to the difficulty in attaining good spray coverage (Byers *et al.* 1984). Similarly, microcapsule formulations have not been applied in tree fruits in spray volumes <500 liters/ha because they are thought to achieve mating disruption by camouflaging the calling of virgin females with a uniform 'fog' of sex pheromone (Doane 1999). Herein, we examine the influence of two spray application techniques on the pattern of deposition of microcapsules and their efficacy in disrupting the sexual communication of codling moth in apple, *Malus domestica* Bordhausen.

MATERIALS AND METHODS

Studies were conducted to compare the efficacy of applications of Checkmate® CM-F (Suterra LLC, Bend, OR), a microencapsulated sex pheromone, with either

an air blast or a low volume sprayer in a 35 yr-old "Red Delicious' apple orchard situated near Naches Heights, WA from 10 July to 08 August 2000. Twelve 0.25 ha plots separated by 75 m were established within the orchard and randomly assigned one of four treatments: air blast application of sex pheromone; air blast application of water as a control; low volume application of sex pheromone; and a low volume application of sex pheromone directly on the ground as a second control. The latter treatment was included to experimentally assess the impact of microcapsules that are not deposited on the foliage following a canopy spray application. Plots consisted of 48 trees (6.8 x 8.0 m tree spacing). Mean $\pm$ SE tree height was 4.3 $\pm$ 0.1 m. Sex pheromone–treated plots were sprayed with a 50:50 mixture of microcapsules containing 14.3% codlemone and microcapsules formulated with 0.50% of a fluorescent material "Dye-Lite" (Tracer Products, Westbury, NY). Codlemone was applied in all plots at a rate of 49.0 g a.i. per ha. A Victair Mistifier (H. F. Hauff Co., Yakima, WA) sprayer with a 100-liter tank pulled by an all-terrain vehicle (ATV) was used to apply the air blast application of sex pheromone and of water alone. Seven spray nozzles angled at 45 $^{\circ}$ and positioned on the sprayer at heights from 1.1 to 1.7 m applied 10.4 liters per minute at 686 kilopascals. Plots were sprayed at a rate of 926 L/ha. The low volume sprayer consisted of a 95-liter polyolefin tank mounted on an ATV. The sprayer was rigged with an adjustable vertical spray boom outfitted with two flat fan nozzles. Nozzles were positioned on the boom at a height of 3.1 m and angled upward at a 45° angle. The two nozzles together deliver a spray volume of 2.36 liters per minute at 18 kilopascals. Plots were sprayed at a rate of 46 L/ha. A horizontal boom attached to the ATV at a height of 1.0 m was used to apply the ground application of sex pheromone and fluorescent materials (49.0 g a.i. per ha) with the same nozzles at the same low volume rate used in the canopy.

The density of fluorescent microcapsules on the ground was estimated by placing five plastic cards (18 x 35 cm) beneath the tree canopy prior to the spray application. Cards were collected < 2 h after sprays were applied, returned to the laboratory, and the number of microcapsules was counted under UV illumination (Black-Ray Long Wave Ultraviolet Lamp, Ultra-Violet Products, Inc., San Gabriel, CA). Cards placed in plots where the material was sprayed directly on the ground were subsampled. Cards were first subdivided into ten 7 x 9 cm squares and the number of microcapsules was counted in one randomly selected square from each card.

The density of fluorescent microcapsules per leaf in the canopy was sampled in both the air blast and low volume-treated plots by collecting five leaves from 10 trees in each replicate from both low (2.2 m) and high (3.2 m) positions in the canopies. Leaves were bagged and microcapsules on both the top and bottom leaf surfaces were counted under UV light in the laboratory.

The efficacy of treatments in disrupting codling moth was evaluated with the use of virgin female-baited traps. Ten wing-shaped sticky traps (Pherocon 1CP, Trécé Inc., Salinas, CA) were placed in each plot spaced approximately 10 m apart and 10 m from the edge. Traps were baited with two virgin female codling moths (< 3 d-old and maintained at 5.0 $\pm$ 0.5 $^{\circ}$C) placed inside a screened PVC cage (4.2 cm [O.D.] x 5.5 cm) hung inside from the top of each trap. Traps were checked and females were replaced every three to four days. The proportion of traps not catching any male moths was used as a measure of mating disruption within each treatment.

Data Analysis. A repeated-measures analysis of variance (ANOVA) was used to compare the proportion of virgin female-baited traps catching male moths with treatment as the between subject factor and week as the within subject factor (Analytical Software 2000). These proportional data were transformed prior to analysis with arcsine (square root [x]). A three-way ANOVA was used to evaluate

the density of microcapsules on leaves with spray application, leaf surface (top or bottom surfaces), and canopy height (low and high) as the main effects. Significant means in all ANOVA's were separated with a least significant difference test, $P < 0.05$. The distribution of microcapsule density between each spray method was compared with a Kolmogorov-Smirnov test (Analytical Software 2000).

RESULTS

Significant differences in disruption of male capture in female-baited traps were found among plots sprayed either with an air blast or a low volume sprayer, plots where the sex pheromone was sprayed on the ground, and unsprayed plots ($F = 6.72$; df = 3, 8; $P < 0.05$) (Table 1). Significant differences were also found in levels of disruption among weeks ($F = 30.84$; df = 3, 9; $P < 0.001$) (Table 1). The interaction of treatment and time was not significant ($P = 0.42$). Disruption of sexual communication was significantly higher in the low volume-treated plots than the other treatments. No difference in the male capture in female-baited traps occurred in plots treated with either the air blast sprayer, applying the sex pheromone directly on the ground, or the application of only water. The proportion of traps catching moths among treatments was significantly lower during the first three weeks of the study versus week four (Table 1).

The density of microcapsules deposited on the ground beneath the trees varied among the three sex pheromone treatments ($F = 78.20$; df = 2, 6; $P < 0.001$). The highest mean $\pm$ SE density of microcapsules was in plots where the sex pheromone was sprayed directly on the ground, 4.5 ± 0.8 per 10 cm^2 and was significantly different from the microcapsule densities on the ground in the canopy-treated plots sprayed with either an air blast, 0.05 ± 0.02 per 10 cm^2 or a low volume sprayer, 0.14 ± 0.05 per 10 cm^2. The density of microcapsules on the ground was not significantly different between the two canopy spray treatments.

The mean density of microcapsules deposited in the lower and upper tree canopy and on the top and bottom surfaces of leaves varied with both spray methods (Fig. 1). The significant three-way interaction for canopy position * leaf position * spray method ($F = 7.61$; df = 1, 16; $P < 0.05$) appears to be the result of low microcapsule deposition on the lower leaf sur-

Table 1.

Mean $\pm$ SE proportion of virgin female-baited traps catching male codling moths in plots treated with microencapsulated sex pheromone (49 g a.i. per ha) applied with either an air blast or low volume sprayer in the canopy or a low volume sprayer on the ground versus water control in replicated 0.25 ha apple plots, July – August 2000.[1]

No. weeks post-spray	Proportion of traps catching moths				Overall week means
	Water control	Air blast canopy	Low volume canopy	Low volume ground	
1	0.30 (0.06)	0.13 (0.07)	0.03 (0.03)	0.30 (0.15)	0.19 (0.05)c
2	0.43 (0.07)	0.43 (0.17)	0.23 (0.03)	0.53 (0.09)	0.41 (0.06)b
3	0.13 (0.13)	0.23 (0.07)	0.03 (0.03)	0.23 (0.03)	0.15 (0.04)c
4	0.90 (0.10)	0.83 (0.03)	0.63 (0.03)	0.70 (0.12)	0.77 (0.05)a
Overall treatment means	0.44 (0.07)a	0.41 (0.09)a	0.23 (0.08)b	0.44 (0.09)a	

[1] Column and row overall means followed by a different letter were significantly different, repeated measures analysis of variance, $P < 0.05$, LSD.

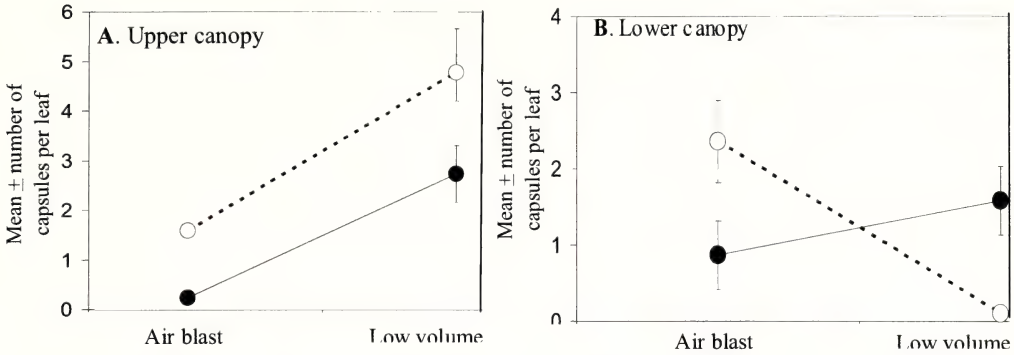

Figure 1. The mean ± SE number of microcapsules deposited on the top (●—●) or bottom (○--○) surface of leaves in the upper (A) and lower canopy (B) of apple trees sprayed with either an air blast or low volume sprayer.

face in the bottom of the canopy when applied with the low volume sprayer (open symbols Fig. 1B). We conducted one-way ANOVA on simple effects means to compare spray methods and to compare leaf surfaces in only the top of the canopy. A significantly greater number of microcapsules were deposited per leaf with the low volume (mean ± SE = 7.5 ± 1.4) versus the air blast (mean ± SE = 3.0 ± 0.7) application in the upper canopy (F = 16.40; df = 1, 4; $P < 0.05$). The difference in microcapsule density was significant between upper and lower leaf surfaces for the air blast sprayer (F = 214.02; df = 1, 4; $P < 0.0001$) with more microcapsules on the lower leaf surface, but not with the low volume sprayer (F=3.69; df = 1,4; P = 0.13).

The overall distribution of microcapsule densities per leaf in the canopy did not differ significantly between spray methods (K-S statistic = 0.10, P = 0.09) (Fig. 2). The highest microcapsule density per leaf in the plots treated with the low volume and air blast sprayer ranged up to 116 and 17 microcapsules per leaf, respectively. Five percent of the leaves sampled in the low volume-treated plots had > 20 microcapsules and these were all in the upper canopy.

DISCUSSION

Spray application method was found to have a significant effect on the efficacy of Checkmate® CM-F for codling moth. An air blast application did not increase the disruption of virgin female-baited traps more than an untreated check or when the material was sprayed directly on the ground. This result is consistent with the poor performance of this formulation in a series of grower field trials using air blast sprayers conducted from 2001-2003 (A. L. K., unpublished data). In contrast, a low volume application to the canopy provided significant levels of disruption during a four-week trial.

The difference in efficacy observed between spray methods was associated with differences in microcapsule deposition. The low volume application deposited significantly more capsules than the air blast spray in the upper canopy. The sexual activity of codling moth is generally restricted to the upper canopy and mating disruption is enhanced when sex pheromone dispensers are placed in the upper versus in the lower canopy (Weissling and Knight 1995). The low volume application also treated 5.0% of leaves with more than 20 microcapsules. The potential for these leaves to serve as attractive point sources and enhance "false-trail following" by male codling moths has not been explored. In general, the emission rate of sex pheromone from individual or clumps of microcapsules has been thought to be too low to create an attractive point source (Sanders

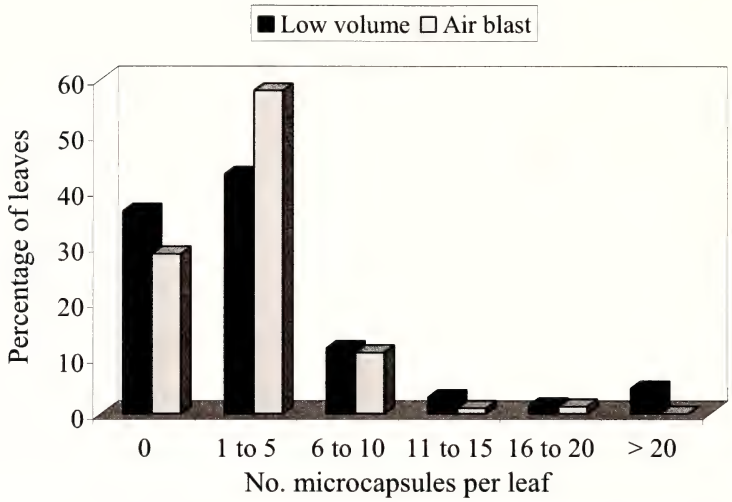

Figure 2. Frequency distribution of microcapsule density per leaf in plots sprayed with either an air blast or low volume sprayer.

1997). Instead, microcapsule formulations are thought to achieve mating disruption by camouflaging the calling of virgin females with a uniform 'fog' of sex pheromone (Doane 1999). However, the mean diameter of the Checkmate® CM-F is approximately 100 µm, which is much larger than many of the formulations (< 5 µm) used in earlier studies (Bakan 1980). Additional research will determine if leaves with variable numbers of microcapsules are attractive to codling moth and whether the low volume application of microcapsules may increase its effectiveness by enhancing the role of "false-trail following".

Factors influencing the deposition of microcapsules due to differences in spray practices are not understood. The two canopy spray methods used in our trials differed in several ways including water volume (20-fold difference) and spray pressure (40-fold difference). However, it is not clear how these factors influence the deposition of microcapsules on foliage. Previous studies have found that the structural integrity of these microcapsules is not impaired by high velocity spray applications (T. E. L., unpublished data). Interestingly, no difference was found in the deposition of microcapsules on the ground beneath trees with either canopy spray application method. The addition of adjuvants could perhaps improve the deposition of microcapsules on leaves with either spray method. Laboratory tests have found that a latex sticker significantly increased deposition and retention of microcapsules on dipped apple leaves before and after simulated rainfall (Knight *et al.* 2004).

The height and angle of the spray nozzle may also affect the deposition pattern within the canopy, i.e., the low deposition of microcapsules on the underside of leaves in the lower canopy in plots treated with the low volume sprayer. Laboratory studies have found that significantly more microcapsules are deposited on the underside versus the top of apple leaves with dipped leaves (Knight *et al.* 2004). This pattern was clearly seen in the upper canopy with the air blast but not with the low volume spray application. Lowering the vertical boom of the low volume sprayer and aiming the spray stream upwards into the canopy would likely increase the deposition of microcapsules on the underside of leaves. Microcapsules deposited on the underside of leaves typically have greater longevity due to shading effects (Hall and Marrs 1989). This may be especially important with conjugated dienes such as codlemone due to their sensitivity to isomerization and oxidation (Millar 1995). The

addition of UV stabilizers has significantly extended the longevity of the Checkmate® CM-F microcapsules; however, the effects of shading provided by the undersurface of apple, pear, or walnut leaves has not been reported (Eng *et al.* 2003).

The compatibility of microencapsulated sex pheromone products with other pesticides has been one factor used to promote their use (Doane 1999). Air blast sprayers apply materials at very high velocities (150 - 300 km/h) to generate a uniform coverage of small droplets throughout the canopy. Most pesticides during the season are applied as concentrated sprays (< 1,000 liters per ha) due to significant reductions in spray costs versus dilute applications (Barnett *et al.* 1991). Yet, this study suggests that applying a sprayable sex pheromone formulation for codling moth is not effective with these standard spraying methods. Difficulties in spraying orchards late in the season with standard equipment could be an important factor limiting the adoption of sprayable pheromone in apple. The low volume sprayer pulled by an ATV allowed orchard rows with a closed canopy to be sprayed without dislodging fruit. The density and deposition patterns of microcapsules should be evaluated with the use of alternative spray methods, such as aircraft, handgun sprayers, or herbicide sprayers. Further reductions in spray volume with these various methods should also be considered.

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Persistence of a commercial codling moth granulovirus product on apple fruit and foliage

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ABSTRACT

Codling moth, *Cydia pomonella* (L.), larval bioassays were carried out on apples and leaves collected from trees treated with the commercially available codling moth granulovirus, Virosoft CP4®, to estimate the persistence of the product over time. The virus had a significant effect on survival of laboratory derived codling moth larvae placed on apples collected up to five and eight days post-treatment. Larvae died with virus symptoms after feeding on treated foliage and the leaf bioassay was easier to count than the apple bioassay. A combination assay, exposing larvae to leaf discs and fruit may more accurately account for potential exposure of wild neonate codling moth to virus in treated orchards. The addition of fish, soybean or mineral oils to Virosoft CP4® treatments did not significantly increase the efficacy or persistence of the viral insecticide on apples in this study.

Key Words: Virosoft CP4®, leaf discs, *Cydia pomonella*

INTRODUCTION

The codling moth granulovirus (CpGV) (Baculoviridae) is found in wild and colonized codling moth (*Cydia pomonella* (L.), Lepidoptera: Tortricidae) (Tanada 1964; Eastwell *et al.* 1999) which is a major pest of apples and pears throughout most of the temperate world (Cross *et al.* 1999). CpGV is noted for its high virulence when ingested by this host, particularly in the neonate stage (Sheppard and Stairs 1976; Tanada and Leutenegger 1968). Commercial formulations of the virus have been registered for use against the codling moth in Europe since 1988 and in the U.S.A. since 1995. In 2000, Virosoft CP4®, produced by BioTepp Inc., Quebec, became registered for use on apples and pears in Canada.

Commercial formulations of CpGV require application of aqueous suspensions of the virus onto the apples and foliage of treated trees. There is a relatively short time when a wild codling moth can be effectively exposed to CpGV treatment in an orchard. Most wild codling moth eggs are oviposited on leaves (Jackson 1979). Neonates move over leaf surfaces before finding a fruit and chewing through the surface where they remain, feed and develop through to the last instar. Ballard *et al.* (2000) found that CpGV was ingested by codling moth neonates browsing on CpGV treated leaf surfaces, therefore there is the potential for codling moth neonates to encounter and ingest lethal levels of CpGV on both treated foliage and fruit surfaces.

Orchard trials of various commercial preparations of CpGV have generally shown good early suppression of neonate codling moth (Glen and Payne 1984; Jaques *et al.* 1987). However, like other viral insecticides, the CpGV is susceptible to inactivation and dilution due to temperature, exposure to sunlight, and precipitation (Jaques 1975, 1985; Glen and Payne 1984). CpGV has been found to be 50% inactivated in 2-8 d on apples (Glen and Payne 1984; Jaques *et al.* 1987; Arthurs and Lacey 2004).

The goal of this study was to determine

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the persistence of Virosoft CP4® on apples and foliage under the orchard conditions in the interior of British Columbia (BC), Canada. We also evaluated whether various oils, added to the spray mixture, could increase or extend the duration of the Virosoft CP4®'s efficacy under orchard conditions, by possibly improving coverage and increasing the penetration of the virus through the leaf surface.

MATERIALS AND METHODS

All treatments were applied to a high density (1m tree spacing within rows) orchard of MacIntosh apple trees at the Pacific Agri-Food Research Centre, Summerland, BC.

Virulence of Virosoft CP4 over time on apples and leaves. Two blocks of five rows of high density apple trees were treated with Virosoft CP4® at 239 ml/ha (original preparation: $4x10^{13}$ occlusion bodies/946.34 ml) on 12 June, 2003 using an air-blast sprayer set to deliver a volume of 2,347.6 L/ha. The Virosoft CP4® was stored refrigerated. An untreated block of trees separated by $\geq$ 30 trees in the same orchard was used as the control. Two hours after application of the virus, 10 leaves and apples were randomly collected from the treatment and the control. Similar collections were made 1, 4, 6, 8, and 12 days after application of the treatments. The entire trial was replicated on different blocks of trees within the same orchard on 3 July, 2003. Mean ± SE daily temperatures during the orchard collection (12-24 June:17.6 ± 3.0 °C; 3-15 July: 22.5 ± 2.3 °C) and the accumulated daily rain that occurred during the June (0.6 ± 1.1 mm/d) and July replications (0 ± 0 mm/d) were similar to daily temperatures (18.8 ± 4.1 °C) and precipitation (0.7 ± 2.3 mm/day) for this area, from 1 June to 15 July, averaged over a 5 y period (1999 - 2004) (Anon. 2004).

Codling moth neonates used in the assays emerged on the same day that the leaves and apples were collected, from egg sheets obtained from the colony of the Okanagan-Kootenay Sterile Insect Release Program (Osoyoos, BC). Five neonate codling moth were placed on the stem end of each collected apple. The apples were sealed in plastic cups and incubated at 24 °C, 16:8 h L:D before codling moth survival was assessed by cutting open the fruit on days 7 and 14. The total number of live larvae in the apple bioassays was a more accurate assessment of the impact of the virus than percentage mortality as larvae occasionally could not be found. Some neonates naturally move off the apple and die by dessication without feeding in apple assays (Laing and Jaques 1980); others disintegrate beyond recognition due to a viral infection or are difficult to locate.

One disc (1 cm diameter) was cut from each leaf, avoiding the center vein, within an hour of their collection and placed on a layer of stikem (Stikem Special™, Phero-Tech Inc., Delta, BC) in a small plastic petri dish. Ten codling moth neonates were placed on each leaf disc. The discs, with the larvae, were placed in the dark for 60 minutes at 22 ± 1 °C to encourage feeding. Larvae that had consumed leaf material frequently had green coloured alimentary canals, green frass and a portion of the leaf surface was scarred. Five larvae which showed evidence of feeding per leaf were transferred to the surface of a pinto-bean based diet (modified from Shorey and Hale 1965) within individual 30-ml plastic cups. The cups were sealed and incubated at 24 °C, 16:8 h L:D until the number of living larvae was assessed on days 7 and 14. Data were analysed using an ANOVA (SAS 2000) and the means were separated within each day the apples or leaves were collected with individual ANOVAs and Tukey's studentized range test.

Virosoft CP4 + oils. In a separate experiment, four blocks of four rows of high density apple trees were each treated with Virosoft CP4® alone at 239 ml/ha (original preparation: $4x10^{13}$ occlusion bodies/946.34 ml), Virosoft CP4® 239 ml/

ha + mineral oil (Superior 70 oil, United Agri Products, Dorchester, ON) at 2L/ha, Virosoft CP4® 239 ml/ha + fish oil (Crocker's Fish Oil, Inc., Quincy, WA) at 2L /ha or Virosoft CP4® 239 ml/ha + once descummed soybean oil at 2.5 L/ha, 17 June, 2004 using an air-blast sprayer set to deliver a volume of 1,553.6 L/ha. A block of untreated trees separated by >10 trees in the same orchard was used as the control. Ten apples were randomly collected from each treatment and the control at least two hours after application of treatments on day 0, as well as 1, 2, 5, 7, and 10 days post-treatment. The entire trial was replicated on 28 June 2004 on different blocks of trees within the same orchard. Mean ± SE daily temperatures during the orchard collection (17-27 June 2: 22.4 ± 2.5 °C; 28 June to 8 July: 17.3 ± 3.4 °C) and the accumulated rain that occurred during the first (2.1 ± 5.5 mm/day) and second replication (0.8 ± 1.3 mm/day) are similar to mean daily temperatures and precipitation for this area, averaged over 5 y, as recorded above. Bioassays were carried out using codling moth neonates on the collected apples as described above. The number of living larvae were counted seven days post exposure. Data were analysed as described for the previous study.

RESULTS AND DISCUSSION

Efficacy of Virosoft CP4 over time on apples and leaves. The Virosoft CP4® had an overall significant impact on the number of codling moth able to survive the virus treatment, compared to control larvae, when the data were collected both 7 and 14 days post-exposure to field collected apples (7 d: $F_{1, 226} = 77.6$, $P < 0.0001$; 14 d: $F_{1, 226} = 66.7$, $P < 0.0001$). Significantly fewer codling moth larvae survived for seven days in apples from trees treated with the virus, compared to untreated apples, two hours, and 1, 4, and 8 days post-treatment with Virosoft CP4® (Table 1). This significant difference between the number of living larvae in the treated versus control apples was extended to 12 days post-treatment when the data were read on day 14, except in apples collected four days post-treatment (Table 1). The number of live codling moth larvae found both in untreated and treated apples was generally lower over all days, when the assays were read at 14 versus 7 days, due to the difficulty locating larvae in the fruit at the later date. The apples had to be carefully sectioned to find the larvae and long term larval mortality data may have been influenced by decay in the fruit. The disappearance and death of a small number of larvae may be partially due to mortality caused by a colony derived CpGV infection and subsequent disintegration of the dead larvae (personal observation). As this mortality would occur in both treated and control apples any differences in surviving codling moth would be attributable to the treatment. Some additional virus-induced death would be expected to occur between days 7 and 14, however, the poor survivorship of larvae in decaying control apples decreases the value of data obtained at this later date.

Codling moth larvae died after consuming CpGV in leaf disc assays and Virosoft CP4® had a significant impact on the number of codling moth larvae able to survive the virus treatment, compared to control larvae (7 d: $F_{1, 226} = 12.6$, $P < 0.0005$; 14 d: $F_{1, 226} = 7.5$, $P < 0.0007$). Significantly fewer living codling moth larvae were found when larvae fed on leaf discs collected two hours and one day post-treatment from Virosoft CP4® treated trees, compared to discs from untreated leaves (Table 1). Similar results were found when the number of surviving larvae were counted 7 and 14 days post-exposure. The leaf disc bioassay was easier to count than the apple bioassay as more larvae were found in the former.

The difference in the active persistence of the virus determined in the apple and leaf disc bioassays is probably due to neo-

Table 1.

Mean ± SE number of live codling moth larvae found per apple or leaf disc after 7 and 14 days when neonates were placed on Virosoft CP4® treated or control apples or leaf discs. Replicated twice; n = 10 apples or leaves; 5 codling moth larvae per apple and leaf disc.

Days post-treatment	Mean ± SE number of live codling moth larvae per apple and leaf disc			
	Read 7 days post-exposure		Read 14 days post-exposure	
	Virosoft CP4®	Control	Virosoft CP4®	Control
	Apples			
0 (2 h)	0.9 ± 0.2 a [1]	2.4 ± 0.3 b	0.2 ± 0.1 a	1.6 ± 0.3 b
1	0.7 ± 0.2 a	3.1 ± 0.3 b	0.8 ± 0.3 a	1.7 ± 0.3 b
4	1.6 ± 0.2 a	3.1 ± 0.3 b	1.1 ± 0.3 a	1.9 ± 0.4 a
6	2.6 ± 0.3 a	3.2 ± 0.2 a	0.9 ± 0.4 a	3.0 ± 0.3 b
8	2.5 ± 0.2 a	3.3 ± 0.2 b	1.2 ± 0.2 a	2.5 ± 0.2 b
12	2.8 ± 0.3 a	3.4 ± 0.3 a	2.1 ± 0.3 a	3.0 ± 0.3 b
	Leaf discs			
0 (2 h)	2.9 ± 0.3 a	4.3 ± 0.2 b	2.2 ± 0.3 a	3.6 ± 0.2 b
1	4.2 ± 0.2 a	4.8 ± 0.1 b	3.5 ± 0.3 a	4.2 ± 0.2 b
4	4.3 ± 0.2 a	4.6 ± 0.1 a	3.4 ± 0.3 a	4.0 ± 0.2 a
6	4.8 ± 0.1 a	4.8 ± 0.1 a	4.1 ± 0.2 a	3.9 ± 0.2 a
8	4.5 ± 0.2 a	4.5 ± 0.2 a	4.1 ± 0.2 a	3.9 ± 0.3 a
12	4.6 ± 0.2 a	4.6 ± 0.2 a	4.1 ± 0.2 a	3.9 ± 0.2 a

[1] Means within rows and days post-exposure followed by the same letter are not significantly different ($P > 0.05$), determined with Tukey's studentized range test (SAS 2000).

nates feeding more readily and extensively on the surface of the apple than on the foliage. Glen and Clark (1985) found that 90% of codling moth neonates hatching from eggs on leaves spent more than 10 min on leaves before moving to the fruit. Penetration of fruit by codling moth has been recorded to take 1 to 2.5 h (Geier 1963). As the neonates in our study were held for 60 minutes on a leaf disc before transferring them to the diet, each neonate had a realistic, but limited chance to ingest the virus. Ballard *et al.* (2000) did not observe feeding by codling moth neonates on leaf tissue until neonates were left for 15 minutes. It is also possible that more virus is accumulated near the stem and the calyx ends of apples (Arthurs and Lacey 2004) and many of the codling moth neonates choose these areas of the apple to feed and then penetrate. Most wild codling moth would encounter CpGV on both the leaf and apple surfaces, therefore it would be appropriate to improve these assays by exposing neonates to both treated surfaces to obtain a more realistic assessment of potential mortality in the orchard.

Virosoft CP4 + oils. The virus treatments had a significant effect on survival of codling moth larvae ($F_{4, 25} = 32.2$, $P < 0.001$). Significantly fewer living codling moth larvae were found in apples collected two hours, one day, and five days post-treatment from trees treated with Virosoft CP4® alone, or in combination with any of the oils, than in the control apples. In apples collected two, seven and ten days post-treatment, this difference was not significant (Table 2). Although the mean numbers of live codling moth larvae per apple in Virosoft CP4® treatments in combination with an oil was lower on days 0 and 1, the numbers were not significantly lower than those in the Virosoft CP4® treatment alone.

The virulence of the Virosoft CP4® applied in June and early July, under conditions that are typical for the southern

Table 2.

Mean ± SE number of live codling moth larvae per apple after 7 days when neonates were placed on fruit treated with Virosoft CP4® combined with one of three oils, or nothing. Replicated twice; n = 10 apples; 5 codling moth larvae per apple.

Days post-treatment	Mean ± SE number of live codling moth larvae per apple per treatment				
	Virosoft CP4®	Virosoft CP4® + Superior 70 oil	Virosoft CP4® + Fish oil	Virosoft CP4® + Soybean oil	Control
0 (2 h)	1.1 ± 0.4 a [1]	0.6 ± 0.1 a	0.3 ± 0.2 a	0.3 ± 0.2 a	3.9 ± 0.5 b
1	1.2 ± 0.3 a	0.7 ± 0.1 a	1.1 ± 0.3 a	0.7 ± 0.2 a	3.7 ± 0.5 b
2	1.8 ± 0.5 a	1.8 ± 0.5 a	1.1 ± 0.1 a	1.6 ± 1.0 a	3.5 ± 0.1 a
5	2.0 ± 0.7 a	1.6 ± 0 a	2.0 ± 0.2 a	1.9 ± 0.2 a	3.9 ± 0.4 b
7	2.4 ± 0.7 a	1.9 ± 0.5 a	2.2 ± 0.7 a	2.0 ± 0.1 a	3.1 ± 0.9 a
10	2.0 ± 0.2 a	1.9 ± 0 a	2.3 ± 0.4 a	2.0 ± 0.5 a	3.1 ± 0.4 a

[1] Means within rows followed by the same letter are not significantly different ($P > 0.05$) as determined with Tukey's studentized range test (SAS, 2000).

interior of BC, decreased quickly over time in both trials. In orchard trials in Washington State, Arthurs and Lacey (2000) found a similar decline in activity and estimated a Virosoft CP4® (3.2 oz/ac) half-life on apples to be 3.8 to 8.7 d post June and July treatments, respectively. It is important to recognize that our study was carried out using colony derived larvae and that wild codling moth larvae may feed on treated apple tree leaves and fruit differently under orchard conditions, which may modify the impact of the virus on the host.

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Species of ground beetle (Coleoptera: Carabidae) in organic apple orchards of British Columbia

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ABSTRACT

In a two year study, 14 genera of Carabidae (*Agonum* Bonelli, *Amara* Bonelli, *Anisodactylus* Dejean, *Bembidion* Latreille, *Carabus* Linné, *Harpalus* Latreille, *Lebia* Latreille, *Loricera* Latreille, *Poecilus* Bonelli, *Pterostichus* Bonelli, *Scaphinotus* Dejean, *Stenolophus* Stephens, *Syntomus* Hope and *Trechus* Clairville) represented by 44 species were identified from six commercial organic apple orchards in the southern Similkameen valley in British Columbia, Canada; 13 of these species were not native to the area. The 4,299 specimens were caught in 'ramp' pitfall traps, with the genera *Pterostichus* and *Harpalus* comprising 56% and 43%, respectively. Numbers of Carabidae ranged from 11-21 species per orchard, with their presence detected throughout the collection period.

Key Words: Carabidae, diversity, abundance, organic orchards, British Columbia

INTRODUCTION

In North America there are 168 genera and over 2200 species of ground beetle (Coleoptera: Carabidae), most of which are predaceous as adults and larvae (Arnett 1993). Carabids are common in tree fruit orchards (Edwards 1998) and many other agricultural (Levesque and Levesque 1994; Raworth *et al.* 1997) and natural ecosystems (Brumwell *et al.* 1998; Toft and Bilde 2002). Their function as polyphagous predators within orchards was recognized in the 1800's (Lord 1983), and several species are known to prey on key tree fruit pests (MacPhee *et al.* 1988; Pearsall and Walde 1994; Sunderland 2002). Eight species have been reported to feed on larvae of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), one of the key pests of pome fruit in Canada (Hagley and Allen 1988; Riddick and Mills 1994).

Carabids are also considered good indicators of ecological change in different communities (Niemelä *et al.* 1993; Niemelä and Kotze 2000; Szyszko *et al.* 2000; Holland 2002; Holland *et al.* 2002). Unfortunately, many pesticides induce significant levels of mortality in carabids (Eptsein *et al.* 2000; Smith *et al.* 2000; O'Flaherty 2002) and lower levels of fecundity (H. Goulet, Eastern Cereal Oil Research Centre, Ottawa, Ontario, pers. comm.) thereby impacting their potential for natural biological control in agricultural systems. Despite the significance of carabids in orchard habitats there have been few published studies of species diversity in the 27,000 ha of Canadian tree fruit orchards (Herne 1963; Hagley and Allen 1988; Pearsall and Walde 1995).

Carabids typically are sampled using pitfall traps (Spence and Niemelä 1994; Raworth *et al.* 1997). Bouchard *et al.* (2000), improving on the ramp pitfall trap designed by Bostanian *et al.* (1983), developed a trap with plastic ramps connected to the upper lip of a container which rested on the terrain surface. In Nova Scotia, a study to evaluate this modified version of the ramp trap for diversity studies in orchards (Rigby and Smith 2002) indicated a similar

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capture performance to the conventional pitfall trap, yet expressed several advantages including minimized fouling of samples by decomposing plant material and reduced capture of non-target invertebrates. In this study, we used these ramp traps to document carabid species found on the orchard floor of organically managed fruit orchards in the southern Similkameen valley of British Columbia.

MATERIALS AND METHODS

Ramp traps were placed in six commercial, certified organically managed apple orchards in the southern Similkameen valley in British Columbia (119.736 °W, 49.169 °N to 119.753 °W, 49.182 °N) in each of two years (1999-2000). Each trap consisted of two ramps and one plastic container with a removable snap-on lid (Figure 1). The container measured 115 mm in diam and 85 mm in height. Two rectangular notches (45 mm x 40 mm) were cut in opposite sides of the container to hold the ramps in place at an angle < 15° (after Bouchard *et al.* 2000) and to serve as an entrance into the container. The ramps were made of 0.75 mm thick transparent copolyester sheets with the upper surface textured with Speckle Stone black/grey aerosol paint (Crown North America Professional Products, Vaughan, Ontario, Canada). Ramps were 300 mm long and 300 mm wide at the lower surface, tapering to 40 mm at the container entrance. The 22 mm lateral edges were folded up at a 90° angle from the surface of the ramp, and the upper end had a 20 mm flap folded down to anchor the ramp to the container.

All six orchards were part of an area-wide codling moth control program which used a combination of mating disruption and sterile insect release to suppress moth populations (Dyck and Gardiner 1992). Orchards contained a mixture of apple cultivars (mainly McIntosh, Spartan, Red Delicious and Golden Delicious) and ranged in size from 0.5 to 4.4 ha. The orchard floors consisted of bare soil and/or vegetation (mainly orchard grass and broad leaf weeds) and were mowed routinely throughout the cropping season. In each orchard three ramp traps were placed beneath the tree canopy, 15 to 30 m apart. Samples were collected in a salt water solution (with a droplet of detergent) which was replaced when samples were removed, at 7 - 10 day intervals from 23 April - 19 October 1999, and 19 April - 2 November 2000. Samples were stored in 70% ethanol until carabid specimens were sorted and identified to species level using the keys of Canadian and Alaskan Carabidae developed by Lindroth (1961-1969b). Voucher specimens are currently held in the Insect Reference collection at the Agriculture and Agri-Food Canada, Atlantic Food & Horticulture Research Centre, Kentville, Nova Scotia.

Figure 1. Ramp pitfall trap used for collection of Carabidae in organic apple orchards.

RESULTS AND DISCUSSION

In the two years of study 44 carabid species from 14 genera were identified (Table 1), of which 13 species (30% of total) were non-indigenous. Non-indigenous species represented 74% of the 4299 beetles captured. These results are similar to those found in previous studies from Nova Scotia: Pearsall and Walde (1995) collected 28 species from 13 genera, of which nine species (32%) were non-indigenous; Smith *et al.* (2000) reported more than 40 species from 19 genera collected in orchards with 70% of the total beetles captured being non-indigenous; O'Flaherty (2002) collected 55 species of Carabidae representing 18 genera. In that study, almost 90% of the total beetles caught were from the 16 non-indigenous species (O'Flaherty 2002).

In this survey, carabid diversity ranged from 11 to 28 species per orchard block (Figure 2). The most frequently collected genus was *Pterostichus* Bonelli, with one introduced species, *P. melanarius* (Illiger) accounting for almost 56% of the specimens captured in the two years. The second most frequently collected genus was *Harpalus* Latreille, which represented 43% of total captures. Many species (46%) were represented by fewer than three specimens each (Table 1). Carabid densities in apple orchards in southern Ontario were found to be highest in the late summer and fall (Holliday and Hagley 1979). Our data documents similar trends, however, the majority of carabids collected were of two species, *Harpalus pensylvanicus* (DeGeer) and *P. melanarius* (Figure 3).

Published data are sparse regarding carabid prey species, but it is generally noted that there is a positive correlation between the size of beetles and that of prey attacked (Larochelle 1990; O'Flaherty 2002). Three of the carabid species collected in this study, *Amara aenea* (DeGeer), *Harpalus affinis* (Schrank) and *Pterostichus melanarius*, have been reported as codling moth predators (Hagley and Allen 1988; Riddick and Mills 1994), but the orchards used in this survey had low codling moth densities (J. Cossentine, pers. obs.), so it is unlikely that it was a primary source of food for these carabid species. However, the generalist predatory tendencies of carabid beetles make them beneficial in orchard ecosystems, since other important pest species serve as food sources (Larochelle 1990; Sunderland 2002).

The species composition of carabid beetles within orchards and most ecosystems in North America appears to be changing with the arrival and successful establishment of non-indigenous species. Jarrett and Scudder (2001) list 19 new British Columbia carabid records and indicate that several of these have been introduced into North America, some with disjunct east and/or west coast distributions. Clearly, both Atlantic and Pacific coastlines serve as arrival points for many species (Jarrett and Scudder 2001), of which many are well established (Bousquet and Larochelle 1993). Within 10 years, it appears that native *Pterostichus coracinus* (Newman), reported by Pearsall and Walde (1995) as comprising over 40% of ground beetles in Nova Scotia orchards, has been displaced by the introduced *P. melanarius*. O'Flaherty (2002) failed to collect *P. coracinus*, while *P. melanarius* accounted for over 45% of beetles captured in that study. Similarly in Nova Scotia, it appears that the native *Harpalus pensylvanicus* is being displaced by *H. rufipes*, which accounted for a large proportion of the beetles caught by Pearsall and Walde (1995) and O'Flaherty (2002). Although *Pterostichus melanarius* is common in British Columbia (Table 1; Raworth *et al.* 1997), *Harpalus rufipes* (DeGeer) has not yet been recorded in this province (Bousquet and Larochelle 1993). As such, *H. pensylvanicus* is still the most common representative of this genus in orchards.

As carabids represent a yet unresolved biological control resource in Canadian

Table 1.

The number and percentage of Carabidae captured in ramp traps in six organically managed apple orchards in British Columbia for 1999 and 2000. Species marked with an asterisk (*) are non-indigenous.

Species	1999		2000	
	Total	%	Total	%
**Agonum muelleri* (Herbst)	45	4.14	65	2.02
**Agonum placidum* (Say)	1	0.09	15	0.47
**Amara aenea* (DeGeer)	1	0.09	16	0.50
**Amara apricaria* (Paykull)	2	0.18	64	2.00
**Amara aulica* (Panzer)	–	–	1	0.03
Amara avida (Say)	7	0.64	8	0.25
Amara californica californica Dejean	–	–	4	0.13
Amara cupreolata Putzeys	11	1.01	7	0.22
**Amara familiaris* (Duftschmid)	21	1.93	48	1.49
Amara latior (Kirby)	21	1.93	79	2.46
Amara littoralis Mannerheim	–	–	1	0.03
Amara musculis (Say)	–	–	6	0.19
Amara obesa (Say)	–	–	5	0.16
Amara carinata (LeConte)	1	0.09	2	0.06
**Anisodactylus binotatus* (Fabricius)	23	2.12	64	1.99
Anisodactylus californicus Dejean	–	–	1	0.03
Anisodactylus harrisii LeConte	5	0.46	11	0.34
Anisodactylus nigerrimus (Dejean)	4	0.36	84	2.62
**Bembidion lampros* (Herbst)	59	5.43	70	2.18
**Bembidion quadrimaculattum dubitans* (LeConte)	1	0.09	–	–
Bembidion rupicola (Kirby)	1	0.09	–	–
**Carabus granulatus granulatus* Linné	22	2.02	40	1.25
Carabus taedatus Fabricius	1	0.09	–	–
**Harpalus affinis* (Schrank)	87	8.00	118	3.67
Harpalus carbonatus LeConte	1	0.09	–	–
Harpalus fraternus LeConte	1	0.09	2	0.06
Harpalus herbivagus Say	–	–	1	0.03
Harpalus nigritarsis C.R. Sahlberg	1	0.09	3	0.09
Harpalus pensylvanicus (DeGeer)	283	26.03	472	14.69
Harpalus seclusus Casey	33	3.04	47	1.46
Harpalus solitaris Dejean	–	–	1	0.03
Harpalus somnulentus Dejean	–	–	6	0.19
Lebia viridis Say	1	0.09	–	–
Loricera pilicornis (Fabricius)	2	0.18	5	0.16
Poecilus lucublandus (Say)	–	–	10	0.31
Pterostichus adstrictus Eschscholtz	2	0.18	1	0.03
Pterostichus corvinus (Dejean)	1	0.09	–	–
**Pterostichus melanarius* (Illiger)	446	41.03	1951	60.74
Pterostichus mutus (Say)	–	–	1	0.03
Scaphinotus marginatus (Fischer von Waldheim)	–	–	1	0.03
Stenolophus conjunctus (Say)	–	–	1	0.03
Stenolophus unicolor Dejean	1	0.09	–	–
Syntomus americanus (Dejean)	1	0.09	–	–
**Trechus obtusus* Erichson	1	0.09	1	0.03
Total number captured	**1087**		**3212**	

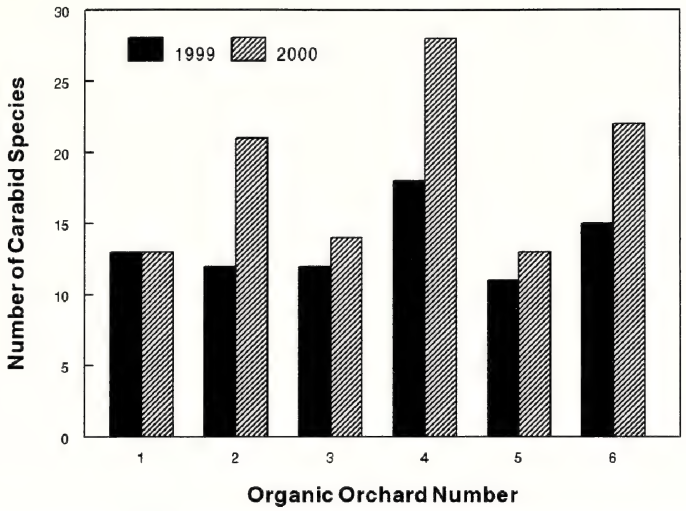

Figure 2. Mean number of Carabidae species captured in ramp pitfall traps in each of six organically managed British Columbia apple orchards in 1999 and 2000.

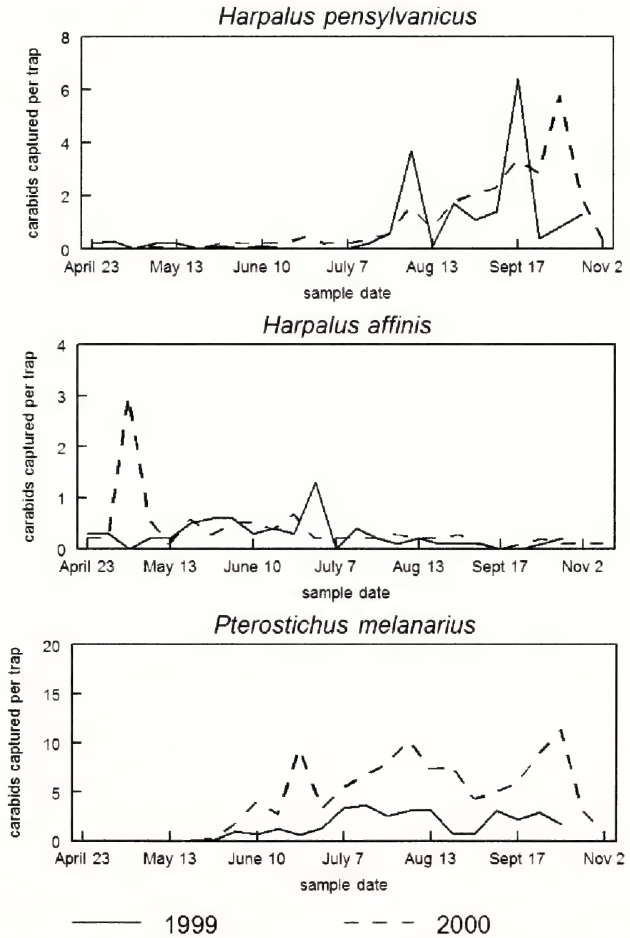

Figure 3. Phenology of three prominent carabid species as monitored by ramp pitfall traps in six organically managed apple orchards in British Columbia: *Harpalus pensylvanicus* (upper graph), *H. affinis* (middle graph) and *Pterostichus melanarius* (lower graph).

orchards, it would be opportune to better understand what they are consuming in the orchards, and how best we can augment their consumption of pest species. This will require further monitoring of carabid populations throughout these ecosystems, and continued assessment of their changing proportions.

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Phenology of emergence from artificial overwintering shelters by some predatory arthropods common in pear orchards of the Pacific Northwest

DAVID R. HORTON[1]

ABSTRACT

The phenology of emergence from artificial overwintering shelters that had been placed in pear orchards located near Yakima, Washington, was determined for the green lacewing *Chrysopa nigricornis* Burmeister, the predatory mirid *Deraeocoris brevis* (Uhler), and the brown lacewing *Hemerobius ovalis* Carpenter. Cumulative emergence from shelters was determined in 2001 and 2002 on both a calendar-date and degree-day basis. Similar data for a major pear pest, pear psylla, *Cacopsylla pyricola* (Förster), were also collected for these same shelters. Pear psylla and *H. ovalis* emerged earliest, both taxa completing emergence by early March (120 degree-days accumulated from early January). *Deraeocoris brevis* emerged beginning in late February and finished emergence by early April (150 degree-days for 90% emergence). *Chrysopa nigricornis* emerged considerably later than the other species, and completed emergence by late May or early June. Calendar-date emergence is also shown for spiders (Araneae) and Anthocoridae (Heteroptera), which occurred at lower numbers in the shelters. The anthocorids, *Orius tristicolor* White and three species of *Anthocoris*, emerged from shelters in February and March, while spiders emerged over a long interval between March and May.

Key Words: overwintering, spring emergence, biological control, pear psylla, green lacewings, brown lacewings, predatory Heteroptera

INTRODUCTION

Many species of predatory arthropods overwinter in pear orchards of the Pacific Northwest (Horton *et al.* 2001, 2002), and it is likely that some of these taxa provide biological control of orchard pests in spring. As broad-spectrum insecticides are used less extensively and selective controls such as mating disruption are put into place, pear growers in the Pacific Northwest may benefit from increased levels of biological control (Knight 1994; Gut and Brunner 1998). Yet, for many predatory taxa, much remains unknown about certain life history characteristics, including diapause, overwintering biology, and post-diapause development. Here, I describe phenology of emergence from overwintering quarters for several predatory species known to overwinter in orchards (Horton *et al.* 2002). Late-winter and early-spring control of pear pests such as pear psylla, *Cacopsylla pyricola* (Förster), is important for season-long management (Westigard and Zwick 1972), and results reported here should assist growers in better predicting when certain predators are likely to be active in their orchards.

MATERIALS AND METHODS

Tree bands of corrugated cardboard were used to provide overwintering shelters for arthropods. Cardboard bands have been used to monitor a variety of overwintering predatory arthropods, including Neuroptera (New 1967; Mizell and Schiff-

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hauer 1987; Horton *et al.* 2002), spiders (Horton *et al.* 2001), and Heteroptera (Fye 1985; Horton *et al.* 2002), all taxa that were monitored in the present study. Each band was 7.6 cm wide and long enough to completely encircle the trunk of the pear tree 0.2 to 0.3 m above the orchard floor. Corrugations in the cardboard were ca. 4 x 5 mm, which is large enough to allow arthropods the size of adult Neuroptera to colonize the corrugations. Ten trees were banded in each of 19 and 24 orchards in October or November of 2000 and 2001, respectively. Orchards were primarily 'Bartlett' and 'D'Anjou' varieties, of various ages. The orchards extended from the eastern Yakima Valley (Zillah and Parker, Yakima County, WA) to the western part of the valley (Yakima, Cowiche, and Tieton, Yakima County, WA). Pest control practices included a range of management programs from conventional insecticidal to organic.

The bands were removed from the field in the first week of January 2001 and 2002 and placed in white plastic boxes (100 x 40 x 24 cm). The boxes had organdy screening on the top and four sides to allow air circulation. A separate container was used for each orchard. Bands and containers were then placed in a large shed enclosed on 3 sides by wire screening, located 15 km southeast of Yakima, WA. The shed was not heated or lighted.

Containers were checked every 3 or 4 days beginning in early January and ending in early June of both years. The lid, sides, and floor of each container were examined for pear psylla, predatory insects, and spiders. Arthropods were counted and aspirated into vials. Specimens other than Chrysopidae and Hemerobiidae were discarded after having been counted. The lacewings were taken to the laboratory for further examination. Chrysopids were identified as *Chrysopa nigricornis* Burmeister using the key in Penny *et al.* (2000). A subsample (n = 42) of Hemerobiidae was examined; all specimens were identified as *Hemerobius ovalis* Carpenter (Kevan and Klimaszewski 1987). Voucher specimens of *C. nigricornis* and *H. ovalis* are in the collection of the author.

Temperature in the shed was recorded at hourly intervals using a Hobo data logger (Onset Computer Corporation, Bourne, MA) placed in one of the containers. In the first year of the study, a second Hobo recorder was placed in a pear orchard located 500 m south of the shed, to determine whether temperatures in the shed were similar to those occurring in the neighboring orchard. The Hobo unit was placed in a white, ventilated wooden box (15 x 30 x 30 cm) at 1.5 m above ground.

Cumulative percent emergence from shelters was expressed as a function of calendar-date and as a function of accumulated degree-days. Emergence curves were developed for the 3 predatory taxa most abundant in the bands, which were two lacewings, *C. nigricornis* and *H. ovalis*, and a mirid bug, *Deraeocoris brevis* (Uhler). One pest species, pear psylla, was also monitored. For some less common taxa (spiders, Anthocoridae), emergence from bands was summarized for two-week intervals and presented in tabular form. "Emergence" used throughout the manuscript refers to the dates that the arthropods were aspirated from the walls of the container. Certain arthropods (e.g., jumping spiders [Salticidae]) may have moved in and out of the corrugations, and for these taxa it is not clear that "emergence", as used here, necessarily reflects what occurs in the field.

Data from the Hobo recorders were used to calculate degree-days by means of a program available on the Oregon State University web-site (Coop 1999). The calculations were done using the single sine curve method and a lower threshold of 5 °C. Accumulations both years began when the bands were placed in the screened shed (8 January 2001 and 10 January 2002).

RESULTS

Temperatures were higher in 2002 than 2001 (Fig. 1). In 2001, when both the screened shed and the neighboring orchard were monitored, temperatures were higher in the shed than in the orchard. Thus, data presented below expressing emergence as a function of calendar-date probably show emergence from bands to have occurred earlier than that taking place naturally in the neighboring orchard.

Pear psylla were extremely abundant in the bands both years (Table 1). Of the natural enemies, *C. nigricornis*, *D. brevis*, and *H. ovalis* occurred at the highest densities. Less common were spiders and Anthocoridae (*Orius tristicolor* (White), *Anthocoris* spp.). *Anthocoris* spp. included *A. antevolens* White, *A. whitei* Reuter, and *A. tomentosus* Péricart. Species' identifications were not made for all samples, so I combined the three species as *Anthocoris* spp.

Pear psylla and *H. ovalis* began emerging from bands by early February in both years of the study (Fig. 2). Psylla had completed emergence by early March (2001) and early February (2002); the difference in calendar dates the two years reflects the warmer temperatures in 2002 than 2001 (Fig. 1). *Hemerobius ovalis* had finished emergence by early March. As with pear psylla, emergence occurred earlier in 2002 than 2001. *Deraeocoris brevis* began appearing in containers in late February both years and had finished emergence by the middle of March in 2002 and early April in 2001 (Fig. 2). *Chrysopa nigricornis* was considerably later in emergence than the other two predatory taxa, and completed emergence by the middle of May in 2002 and by early June in 2001. Emergence in 2001 did not begin until early May; by this time in 2002, *C. nigricornis* had achieved 50% emergence.

Emergence from shelters was also expressed as a function of cumulative degree-days (Fig. 3). Full emergence from shelters by pear psylla required few heat units. *Hemerobius ovalis* had completed 90% of emergence by 73 and 111 cumulative degree-days in 2001 and 2002, respectively. Curves for *D. brevis* were very similar the two years, and this species required about 150 degree-days to complete 90% emergence from shelters (Fig. 3). *Chrysopa nigricornis* began emerging from bands at about 400 degree-days, and required 600 to 730 degree-days to complete 90% emergence. Fifty percent emergence for *C. nigricornis* required 500 and 520 cumulative degree-days in 2001 and 2002, respectively. Curves for *C. nigricornis* were very similar in the two years until at the end of the emergence period, when emergence from bands in 2001 was slower than in 2002 (Fig. 3).

Spiders emerged over a fairly broad interval (Table 2), probably because this taxon comprised a mix of species. The anthocorids emerged in late February and early March (Table 2).

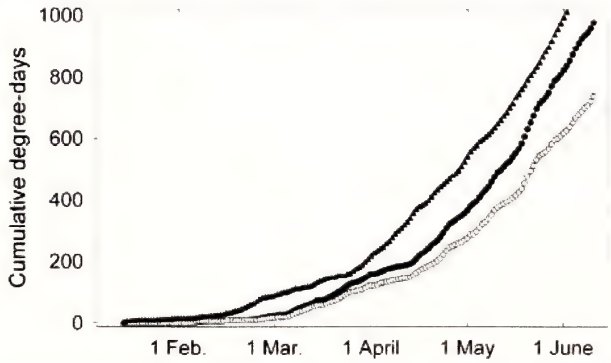

Figure 1. Accumulated degree-days within shed, 2001 (closed circles) and 2002 (closed triangles), and in an orchard neighboring the shed, 2001 (open circles).

Table 1.
Cumulative numbers of common arthropods emerging from cardboard bands in Jan-June of 2001 (N = 190) and 2002 (N = 240).

	2001	2002
Cacopsylla pyricola (Homoptera: Psyllidae)	4638	4778
Chrysopa nigricornis (Neuroptera: Chrysopidae)	237	163
Deraeocoris brevis (Heteroptera: Miridae)	159	235
Hemerobius ovalis (Neuroptera: Hemerobiidae)	136	111
Spiders (Araneae)	42	104
Orius tristicolor (Heteroptera: Anthocoridae)	6	69
Anthocoris spp. (Heteroptera: Anthocoridae)	8	66

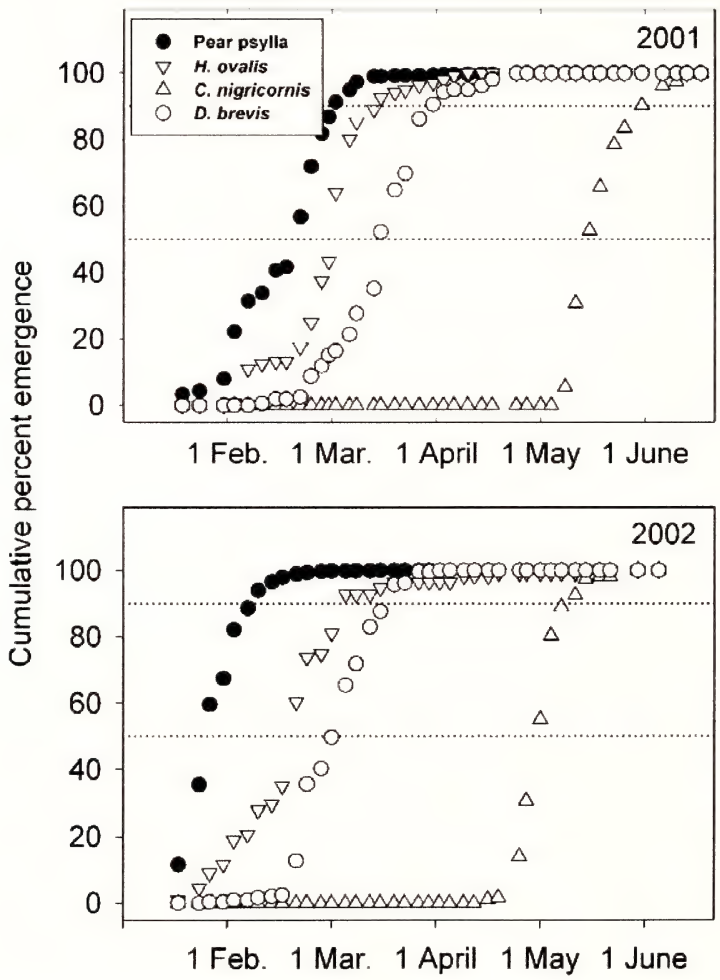

Figure 2. Cumulative emergence from bands in 2001 and 2002 by pear psylla (solid circles), *Hemerobius ovalis* (inverted triangles), *Deraeocoris brevis* (open circles), and *Chrysopa nigricornis* (triangles) versus calendar date (cf. Table 1). Dotted lines depict 50 and 90% cumulative emergence.

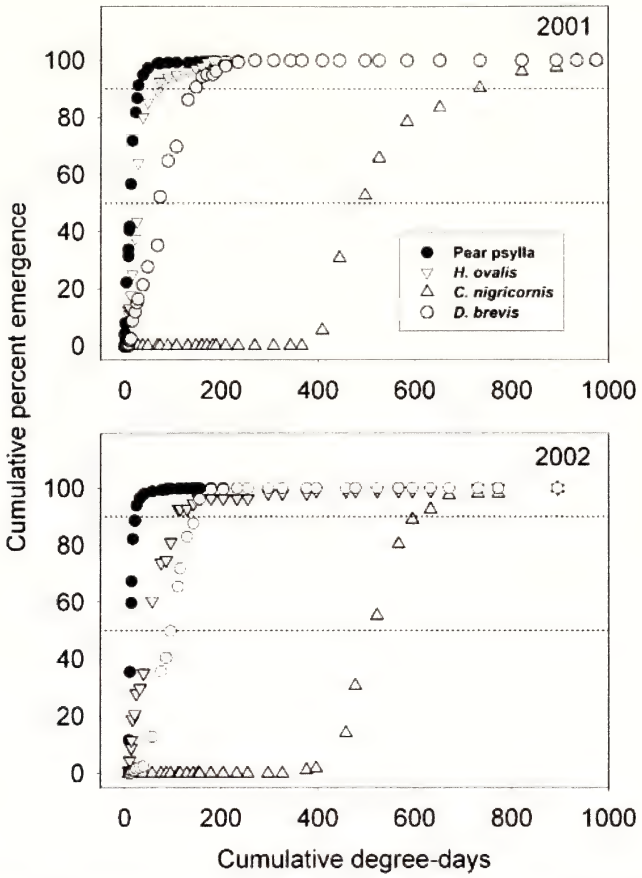

Figure 3. Cumulative emergence from bands in 2001 and 2002 by pear psylla (solid circles), *Hemerobius ovalis* (inverted triangles), *Deraeocoris brevis* (open circles), and *Chrysopa nigricornis* (triangles) versus accumulated degree-days (cf. Table 1). Dotted lines depict 50 and 90% cumulative emergence.

Table 2.

Numbers of spiders, *Orius tristicolor* and *Anthocoris* spp. (*A. antevolens*, *A. whitei*, *A. tomentosus*) emerging from bands in 2001 and 2002 versus calendar date.

	2001	2002		
	Spiders	Spiders	*Orius tristicolor*	*Anthocoris* spp.
1-15 Jan	0	1	3	1
16-31 Jan	0	10	19	7
1-15 Feb	0	7	35	16
16-28 Feb	5	7	10	22
1-15 Mar	8	18	2	9
16-31 Mar	8	25	0	7
1-15 Apr	9	23	0	4
16-30 Apr	6	10	0	0
1-15 May	5	3	0	0
16-31 May	1	0	0	0

DISCUSSION

With reduced use of broad-spectrum insecticides and increased use of selective controls such as mating disruption or narrow-spectrum insecticides, fruit growers in the Pacific Northwest may experience increased levels of biological control in their orchards (Westigard *et al.* 1968; Knight 1994). Results reported here and elsewhere (Horton *et al.* 2001, 2002) indicate that a large diversity of predatory arthropods may overwinter in pear orchards. Horton *et al.* (2001, 2002) described seasonal phenology of autumn entry into overwintering shelters by natural enemies in pear orchards. Practical aims were to tell growers whether late-season insecticide applications, if made, would occur while predatory arthropods were still active in the orchard. In the present study, I examined phenology of emergence from overwintering quarters. Calendar-day or degree-day models for use in predicting emergence from overwintering sites in deciduous fruit or nut orchards in North America have been developed for both pest (Bergh and Judd 1993) and predator (Felland *et al.* 1995; Mizell and Schiffhauer 1987) species.

Of the four most common taxa that emerged from the bands, pear psylla was the most abundant (Table 1). This insect is among the most damaging arthropod pests of pears in North America and Europe (Westigard and Zwick 1972; Solomon *et al.* 1989). Pear psylla overwinters in the adult stage as a distinct morphological phenotype, the winterform, which began appearing in pear orchards of the study area in early September. The species is active at cool temperatures, and can be seen moving about on the pear tree in midwinter on sunny days. Pear psylla began emerging from the bands after only minimal accumulation of heat units (Fig. 3). Egg-laying in the study area commences by March (Horton 1999).

A green lacewing, *C. nigricornis,* was also abundant both years in the bands (see also Horton *et al.* 2002). This green lacewing is a common inhabitant of deciduous fruit and nut orchards (Grasswitz and Burts 1995; Szentkirályi 2001), apparently as a generalist predator of soft-bodied insects such as aphids, mealybugs, and psyllids (Toschi 1965; Grasswitz and Burts 1995); I have reared *C. nigricornis* to the adult stage on a diet of nymphs and eggs of pear psylla (DRH, unpublished data). *Chrysopa nigricornis* has a facultative diapause controlled by photoperiod, and overwinters as a third instar within the cocoon (Tauber and Tauber 1972). The species emerged from overwintering shelters considerably later in the season than the other predator or pest species monitored here, and had not completed emergence until well into May both years (Fig. 2). Mizell and Schiffhauer (1987) showed that *C. nigricornis* emerged from overwintering shelters placed in pecan orchards of Georgia beginning in late March and early April, much later than spiders and a coccinellid beetle common in those orchards.

The brown lacewing *H. ovalis*, like pear psylla, began emerging from bands after only minimal accumulation of heat units (Fig. 3). Species of Hemerobiidae often show activity or development at relatively cool temperatures (Neuenschwander 1976; Canard and Volkovich 2001), so their emergence in late winter is not unexpected. Biology of *Hemerobius* species, including diapause and overwintering, is poorly described. Within the Hemerobiidae, all stages from egg to adult have been recorded to overwinter (Canard and Volkovich 2001). In the present study, brown lacewings emerged from the bands as adults, and I assume that they overwintered in this stage. Adults of several *Hemerobius* species, including *H. ovalis*, have been collected in mid-winter in areas of the Pacific northwest (Foster 1942; Kevan and Klimaszewski 1987), which is additional evidence that this species overwinters in the adult stage. The role of brown lacewings in orchards has not been systematically studied, despite their fairly

regular appearance in temperate zone orchards (Szentkirályi 2001). McMullen and Jong (1967) reported that *H. pacificus* Banks preyed upon eggs and nymphs of pear psylla in Canadian pear orchards, while Nickel *et al.* (1965) stated that *H. angustus* Banks provided biological control of psylla in California pear orchards.

Deraeocoris brevis has a facultative diapause controlled by photoperiod, overwintering in the adult stage (Horton *et al.* 1998). This species emerged from bands beginning in late February or early March, and had completed emergence by mid- to late-March. *Deraeocoris brevis* preys extensively on soft-bodied arthropods such as aphids and psyllids (McMullen and Jong 1967; Messing and AliNiazee 1985) and occupies a variety of habitats including pear and apple orchards (Horton and Lewis 2000). When pear psylla is at high densities in pear orchards, this predator may be among the most abundant of natural enemies in the orchard (Westigard *et al.* 1968). McMullen and Jong (1967) stated that *D. brevis* is second in importance only to species of *Anthocoris* as a predator of pear psylla in pear orchards of the Pacific northwest.

Maximal use of natural enemies in orchards requires improved understanding of natural enemy biology, including overwintering biology. Horton and Lewis (2000) described use of natural habitats and orchards for overwintering by various species of predatory arthropods, while Horton *et al.* (2001, 2002) described late-season phenology of predators entering overwintering shelters. By knowing when predators emerge from overwintering quarters, growers would have a better idea about the potential for biological control early in the season, a critical time for managing pests such as pear psylla. Results reported here suggest that at least one known important predator of pear psylla, *D. brevis*, emerged from shelters early enough in late winter that it could be active in pear orchards at the same time that overwintered pear psylla would be depositing substantial numbers of eggs (March and April). The data also suggest, however, that *D. brevis* and several other taxa (*H. ovalis*, *O. tristicolor*, *Anthocoris* spp.) could be active in Pacific Northwest orchards at the time of the earliest insecticide sprays. Thus, it seems possible that these predators could be exposed to those early-season pest control practices.

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Douglas-fir beetle lipid levels in relation to tree physical characteristics

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ABSTRACT

The relationship of Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, brood adult lipid levels and position of development along infested tree boles was investigated. In addition, the effects of phloem and bark thickness on brood adult lipid levels were also tested. There were no significant differences ($P > 0.05$) in brood adult lipid levels in relation to bole position, phloem thickness, or bark thickness found in this study. Numbers of attacks, larval mines, brood adults, and parasitoid cocoons did not differ significantly by tree bole position. Results from this study suggest Douglas-fir beetle does not benefit, in the form of increased lipid levels, from oviposition at different bole positions.

Key Words: *Dendroctonus pseudotsugae*, lipids, phloem thickness, optimal habitat

INTRODUCTION

Bark beetles are economically important insects and knowledge of factors that affect their flight and dispersal behavior could be useful for improving existing management techniques or developing new ones. Within a population beetles display varying degrees of flight capabilities from extended flight periods to those incapable of flight (Atkins 1966; Jactel 1993). This variation in flight capability can be related to a beetle's physiological state.

Lipids are a source of energy for insect flight (Canavosa *et al.* 2001) and have been correlated with flight capabilities in bark beetles (Atkins 1966; Slansky and Haack 1986; Jactel 1993). Atkins (1966) found that Douglas-fir beetle (DFB), *Dendroctonus pseudotsugae* Hopkins, with high lipid levels were least likely to respond to pheromones and hence disperse, while beetles with low lipid levels responded immediately to pheromones. Bennett and Borden (1971) found that a 90 minute flight was required before DFB responded to pheromones, suggesting the need to metabolize lipids before pheromone arrestment occurred (Atkins 1969). Relationships between lipid levels and responsiveness to host chemicals, pheromone arrestment, or dispersal behavior have been found in other bark beetle species as well (Hagen and Atkins 1975; Hedden and Billings 1977; Wallin and Raffa 2000). Because of their association with bark beetle dispersal potential, a better understanding of factors that influence lipid levels is important for understanding population movements.

Bark beetle lipid levels are influenced by temperature (Atkins 1967), attack density (Atkins 1975; Botterweg 1983; Anderbrandt *et al.* 1985), mycangial fungi (Coppedge *et al.* 1995) and phloem thickness (Slansky and Haack 1986). However, with the exception of *Ips calligraphus* (Germar) (Slansky and Haack 1986), it is unknown whether host tree characteristics affect lipid levels in bark beetle brood adults. Nutrient levels (N, P, Mg, Fe, Zn) vary by bole height on Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, tree boles and could influence patterns of insect colonization (Schowalter and Morrell 2002). In several *Dendroctonus* species,

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initial attacks occur at or near mid-bole (Miller and Keen 1960; Fargo *et al.* 1978; Safranyik *et al.* 1992) possibly due to phloem nutrients at these heights. Consequently, brood developing at mid-bole could have higher lipid levels than brood developing elsewhere along the tree bole.

Other factors, such as parasitism or predation rates, could influence colonization behavior. Studies investigating the relationship between parasitoid density and tree height have produced mixed results. Several studies found relationships between parasitoid density and height on tree boles (Ryan and Rudinsky 1962; Mills 1986; Wermelinger 2002), while others have not (Gargiullo and Berisford 1981).

Our objectives were to determine if DFB brood development position along the length of tree boles and bark and phloem thickness affected lipid levels in brood adults and to determine the influence of bole position on attack density, larval mines, brood adults, and parasitoids.

MATERIALS AND METHODS

Tree Sampling. On 27 to 29 April, 2002, prior to the DFB flight period, nine Douglas-fir trees infested the previous year were felled and sampled from a small stand (< 1 ha) of pure Douglas-fir in the Rock Creek Area (N 46° 34.619' W 113° 40.067'), 60 km southeast of Missoula, MT. The sample trees ranged in diameter from 40.2 to 71.6 cm. An additional six trees were felled and sampled from one Douglas-fir stand on the Flathead National Forest (N 46° 25.316; W 114° 37.995) near Whitefish, MT on 6 April, 2003. However, because DFB attacks were only successful on small portions of the tree boles, only two of the six additional trees (41.1 and 53.3 cm diameter breast height) were suitable for use in this study. Pheromone baiting live trees prior to DFB flight was considered, but concerns over initiating large infestations on federal lands and the unnatural selection of host trees necessitated avoidance of this method. All sample trees in this study were naturally selected and colonized by DFB.

The portion of each tree bole infested by DFB was distinguished by the presence of successful egg galleries and brood adults. Total length of infested tree boles and dbh were recorded. Infested tree bole lengths ranged from 6.7 to 14.6 m ($\bar{x}$ = 9.7 m, SE ± 0.8). Bark samples were collected at three positions along the infested tree bole: 2 m up from the bottom of the infestation, the mid-point of the infested tree bole, and 2 m down from the top of the infestation.

Four bark samples were collected from each bole position. Bark samples were taken randomly around the circumference of each tree. A 100-cm^2 hole saw attached to a power drill was used to remove bark samples from the infested tree. Bark samples were removed and placed individually in labeled plastic bags. Samples were transported to the laboratory on ice and stored in a freezer at -10 °C until processed. Phloem thickness was measured on a subsample of bark samples before and after freezing. No differences in the average phloem thicknesses before and after freezing were found (KJD, unpublished data).

Bark Analysis. Brood adults were removed from bark samples and placed individually in numbered 7 ml glass vials with caps attached. Numbers of DFB entrance holes, larval mines, parasitoid cocoons, and bark and phloem thickness were recorded for each bark sample. Bark thickness was measured on four locations around each bark sample, while phloem thickness was measured in two locations. To account for bark thickness variability, the minimum and maximum thicknesses on each sample were recorded along with two random measurements.

Lipid Analysis. Beetles (n = 283 from 2002, 73 from 2003) removed from bark samples were placed in an oven to dry at 70 °C for 48 h then weighed. To determine the lipid levels of individual beetles,

petroleum ether was used to remove lipids with methods modified from Langor *et al.* (1990). Briefly, 5 ml of petroleum ether was added to each vial, the vial was capped, and then placed in a drying oven at 50 °C for 24 h. Petroleum ether was removed and replaced with fresh solvent every 24 h for a total of 72 h. After the extraction was complete, beetles were oven dried for 48 h and reweighed. To ensure all lipids were extracted, dried beetles were again placed in petroleum ether for 24 h, oven dried for 48 h, and reweighed. Because there was no change in their extracted weights, it was assumed all extractable lipids were removed from the beetles during the initial 72-h process. Lipid levels were calculated as percent loss in dry weight. Beetles that had 0% lipid levels were assumed dead at the time of sampling and discarded from the study.

Gender of each DFB brood adult was determined (Jantz and Johnsey 1964). In addition, pronotal width of each beetle was measured using microcalipers and a dissecting microscope.

Statistical Analysis. Analyses of variance (PROC MIXED, SAS 8.0) were conducted with trees as blocks and bark and beetle samples grouped by bole position to test for differences in bark and phloem thickness, numbers of entrance holes, brood adults, parasitoid cocoons, and larval galleries by height. Residual and normality plots were visually interpreted for homogenous variances and normality. Where necessary, data were log transformed to meet ANOVA assumptions of normality or variability. Means were compared and separated using Tukey pairwise comparisons. Regression analysis was used to determine if there was a relationship between the average lipid levels and number of brood adults found in each bark sample. In addition, relationships between bark and phloem thickness, and lipid levels and pronotal widths were analyzed using analysis of covariance. Analysis of covariance was also used to investigate the relationship between parasitoid abundance and bark thickness. Differences in lipid levels and pronotal width by gender were assessed using ANOVA. All reported means and confidence limits were backtransformed from data used in the statistical analyses.

RESULTS

There were no significant differences between brood adult lipid levels and bole position ($F_{2, 20} = 0.75$, $P = 0.48$). Overall lipid levels ranged from 3.12% to 43.08% and averaged 21.3 (95% CL = 17.8-25.5), 23.6 (19.5-28.2), and 21.8 (18.0-26.3) for the bottom, middle, and top position respectively. Less than 26% of beetles from both sample years had ≤ 15% lipid levels (Figure 1). The largest number of beetles from all three bole positions had between 25-35% lipids, while very few had ≤ 5% lipids (Figure 2). Analysis of covariance indicated there was no significant relationship between phloem thickness ($F_{2, 20}= 0.87$, $P = 0.43$) or bark thickness ($F_{2, 20}= 0.73$, $P = 0.49$) and lipid levels. Phloem thickness ranged from 1.5 mm to 4.9 mm, while bark thickness ranged from 7.5 mm to 32.6 mm. There were no significant relationships between pronotal width and bole position ($F_{2, 20}= 2.02$, $P = 0.16$), phloem thickness ($F_{2, 20}= 1.89$, $P = 0.18$), or bark thickness ($F_{2, 20}= 0.34$, $P = 0.72$).

Phloem thickness, numbers of entrance holes, larval mines, brood adults, and parasitoid cocoons were not significantly different among the three bole positions (Table 1). However, bark thickness was significantly different among the three heights ($F_{2, 20}= 32.74$, $P < 0.0001$). Bark at the bottom was thicker than the middle ($t_{20} = 4.95$, $P = 0.0002$) and top ($t_{20} = 8.02$, $P < 0.0001$) of tree boles, while the middle was also thicker than the top ($t_{20} = 3.07$, $P = 0.02$). There was no significant relationship between lipid levels and number of brood adults in bark samples ($F_{1,105}= 1.44$, $P = 0.23$). Analysis of covariance indicated there was no relationship between

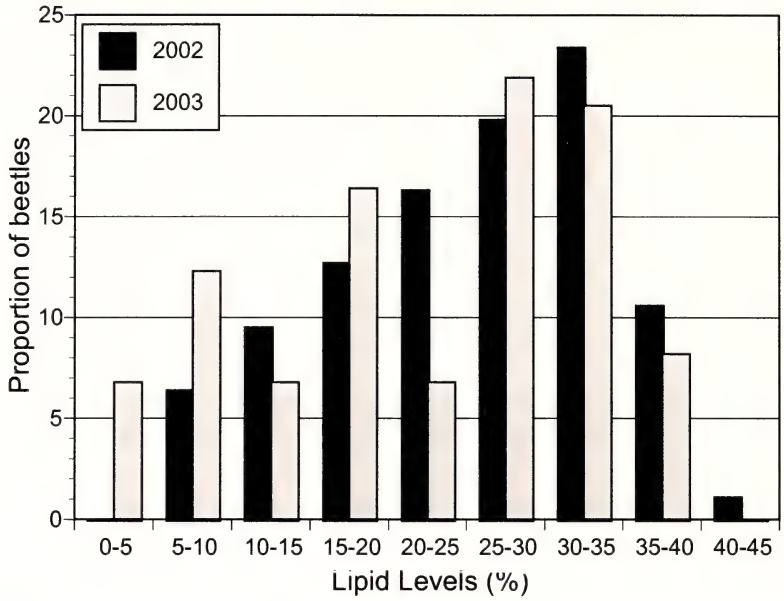

Figure 1. Frequency distribution of the proportion of Douglas-fir beetle brood adults by lipid levels (%) in 2002 (n = 283) and 2003 (n = 73).

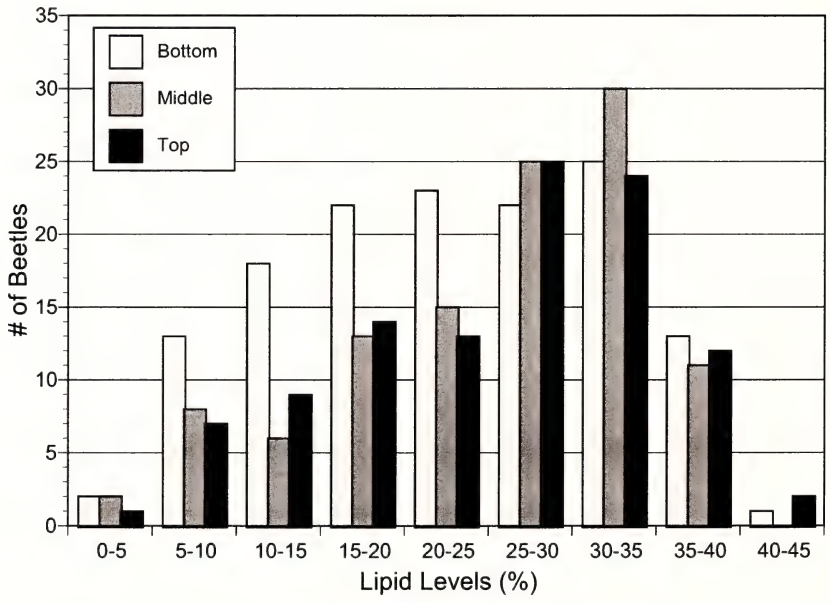

Figure 2. Frequency distribution of Douglas-fir beetle brood adult lipid levels (%) by bole position. n = 356.

number of parasitoids and bark thickness ($F_{2, 20}$= 0.92, P = 0.42).

Overall, the gender of brood adults was 55% female and 45% male. Average brood adult lipid levels were significantly higher in female (25.3%, CL = 22.4%, 28.2%) than in male (23.0%, CL = 20.1%, 26.0%) DFB ($F_{1, 322}$ = 6.88, P = 0.009). There was no difference in pronotal width between male and female DFB ($F_{1, 318}$ = 0.16, P = 0.70).

Table 1.
Mean (95% CL) bark and phloem thickness and Douglas-fir beetle and parasitoid population parameters from 100-cm^2 bark samples taken at three bole positions. Means followed by the same letter are non-significant as determined by Tukey's pairwise comparisons ($\alpha = 0.05$). Eleven trees were sampled, with a total of n = 44 bark samples taken from each of the three bole position.

	Bole Position			
Variable	**Bottom**	**Middle**	**Top**	***P*-value**
Bark (mm)	16.28 (13.87-18.91)a	13.74 (11.82-16.12)b	12.43 (10.69-14.44)c	<.0001
Phloem (mm)	3.49 (3.22-3.82)	3.39 (3.1-3.71)	3.39 (3.1-3.71)	0.54
No. entrance holes	0.73 (0.55-0.92)	0.91 (0.72-1.09)	0.8 (0.62-0.98)	0.40
No. brood adults	3.49 (2.56-4.81)	2.64 (1.95-3.6)	2.77 (2.03-3.78)	0.29
No. parasitoid cocoons	1.36 (1.08-1.7)	1.32 (1.06-1.65)	1.58 (1.27-1.97)	0.41
No. larval galleries	24.95 (17.31-32.6)	31.14 (23.49-38.78)	27.36 (19.72-35.01)	0.11

DISCUSSION

Intraspecific competition has been correlated with lipid levels in DFB and other bark beetle species, (Atkins 1975; Botterweg 1983; Anderbrant *et al.* 1985), and must be considered when evaluating factors that influence brood adult lipid levels. Because entrance holes, larval mines, and brood adult densities were equal at the three bole positions, it was assumed developing brood encountered similar intraspecific competition levels at each position. Therefore, intraspecific competition should not have influenced lipid levels in this study.

Lipid level was not influenced by bole position or phloem thickness in this study. However, unmeasured factors such as phloem quality (e.g., nutrient level), gut flora, genetics, or length of feeding could also influence lipid levels. Ayres *et al.* (2000) found a positive relationship between *Dendroctonus frontalis* Zimmermann size and phloem nitrogen levels of infested trees, demonstrating that phloem qualitative characters determine fitness attributes of phloem-feeding insects. Phloem nutritional levels at the three bole positions were not sampled in this study because sampled trees were already colonized and extensively fed upon by bark beetle brood and associated insects (e.g., Cerambycidae and Buprestidae) at the time of sampling.

There was no relationship between bark thickness and lipid content of DFB. Bark thickness imparts some level of insulation on host tree phloem (Graham 1924; Beal 1934; Powell 1967) and influences bark beetle brood survival during cold periods (Miller and Keen 1960). Consequently, bark thickness may have influenced lipid levels in this study, but differences may have been undetected due to the sampling procedure. Bark samples were not partitioned by cardinal direction, thus aspect-related differences in lipid levels could not be analyzed.

Female brood adults had higher lipid levels than males in sample trees, while pronotal widths were equal between the sexes. In *Dendroctonus* species, females locate and initiate colonization of host trees. This supports the hypothesis that higher energy levels benefit dispersing females that must locate, colonize, and release aggregation pheromones that attract conspecifics to overwhelm host tree defenses. Similarly, Anderbrant *et al.* (1985)

found higher lipid levels in male *Ips typographus* (L.) and attributed this to males being the colonizing sex, and therefore benefiting from increased energy reserves.

Atkins (1966) determined that DFB adults with lipid levels of less than 10% were unlikely to fly, those with 11-20% lipid content can fly and respond to pheromones, and brood adults with over 20% lipid content disperse but are less likely to respond to pheromones. In the current study, the majority of beetles from both sample years had > 20% lipids (Figure 1). Based on Atkins (1966) data for potential to disperse, 67.7% of beetles sampled in this study, regardless of the bole position where they developed, would be capable of long distance dispersal.

There were no differences between parasitoid densities at each bole position. Although bark was significantly thinner at the upper bole position, an attribute commonly associated with higher parasitism levels, there was not a higher level of parasitoid abundance found there. Likely, parasitoids are exploiting thin bark portions or bark crevices at all positions.

Bark beetles colonizing host trees are affected by natural predators (Reeve 1997; Aukema and Raffa 2002), competitors (Schroeder and Weslien 1994; Dodds *et al.* 2001), and host tree defenses (Raffa and Berryman 1983). In addition to minimizing interactions with these mortality factors, bark beetles must also locate areas that are suitable for brood development and reproductive success. If lipid levels are viewed as a relative fitness measure, there seems to be no benefit to oviposition on different bole positions for developing DFB brood. Consequently, it is unlikely that the colonization behavior of attacking the mid-bole first, is a fitness response to seeking out and exploiting optimal habitat for developing brood. While no relationship between lipids and bole position were found, other factors (e.g., avoidance of predators or host tree defenses) might make oviposition at the mid-bole beneficial.

Results from this study suggest DFB brood adult lipid levels are not influenced by tree phloem or bark thickness. However, due to low DFB populations in the study area, the number of infested trees available to sample was lower than anticipated. Consequently, several of the response variables measured had wide 95% CL ranges that may have been smaller had more trees been available to sample. If the number of sample trees had been increased, differences in lipid levels between the three bole positions may have been detected.

Beetles emerging from different host trees within or between forest stands could explain the population level variations in brood adult lipid levels and subsequent flight behaviors found in wild populations. Further studies into the direct relationship of phloem characteristics and lipid levels may help explain landscape level dispersal behaviors of DFB and mortality patterns attributed to this beetle.

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Evaluation of two repellent semiochemicals for disruption of attack by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae)

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ABSTRACT

When released from attractant-baited multiple-funnel traps, 3-methyl-2-cyclohexen-1-one (MCH) reduced catches of male and female mountain pine beetles, *Dendroctonus ponderosae* Hopkins, by 67.4% and 71.8%, respectively. 2-Phenyl ethanol reduced the respective catches by 96.6% and 95.1%, but only verbenone and all three compounds together reduced catches to levels no different from those in unbaited control traps. In another experiment, all three binary combinations of the above compounds, plus the ternary combination, reduced catches of both sexes by >96%. In comparable tree protection experiments near Princeton BC, MCH and 2-phenyl ethanol alone and together significantly reduced the percentages of pheromone-baited lodgepole pines that were attacked by 16.0%, 33.3% and 40.0%, respectively, but verbenone alone totally protected baited trees, and many trees within 5 m of them, from attack. In identical experiments near Prince George BC, where mountain pine beetle populations were much higher, adding MCH, 2-phenyl ethanol or both together to verbenone did not cause attack to be reduced significantly beyond that achieved by verbenone alone. Our results confirm that 2-phenyl ethanol is an antiaggregation pheromone for the mountain pine beetle, and that MCH is an interspecific synomone. However, because neither was as effective as verbenone in protecting pheromone-baited trees from attack, and adding either or both to verbenone did not improve protection, neither compound warrants further consideration as a potential tool for operational disruption of attack.

Key Words: *Dendroctonus ponderosae*, semiochemicals, pheromones, verbenone, 2-phenyl ethanol, 3-methyl-2-cyclohexen-1-one, attack disruption

INTRODUCTION

Although the antiaggregation pheromone verbenone has long been known to disrupt attack by the mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins (Amman *et al.* 1989; Lindgren *et al.* 1989a), its efficacy has been inconsistent between years, target species of trees, and geographic areas (Bentz *et al.* 1989; Lister *et al.* 1990; Gibson *et al.* 1991; Shea *et al.* 1992). Part of the reason for variable efficacy may be that verbenone is transformed to the inactive compound chrysanthenone when exposed to ultraviolet radiation (Kostyk *et al.* 1993). Adding repellent nonhost volatiles from angiosperm tree bark to verbenone has been shown to increase the efficacy of protecting lodgepole pines, *Pinus contorta* var. *latifolia* Engelmann, from attack (Huber and Borden 2001), and combining a seven-component nonhost

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volatile blend with an increased release rate of verbenone has raised the efficacy even higher (Borden *et al.* 2003). However, at an effective 10 x 10 m spacing, the latter treatment would cost $1,250 per ha, excluding labor, limiting its potential use.

One means of reducing the cost would be to replace the repellent nonhost volatile blend with cheaper materials. Two such semiochemicals are the antiaggregation pheromone 2-phenyl ethanol (Pureswaran *et al.* 2000) and 3-methyl-2-cyclohexen-1-one (MCH). MCH is an antiaggregation pheromone of Douglas-fir and spruce beetles, *Dendroctonus pseudotsugae* Hopkins and *D. rufipennis* (Kirby), respectively (Rudinsky *et al.* 1972; Lindgren *et al.* 1989b) that was recently shown to be a repellent synomone for the mountain pine beetle (Pureswaran and Borden 2004). Our objectives were to confirm the bioactivity of 2-phenyl ethanol and MCH, and to determine in trapping and tree protection experiments whether they are potential adjuvants that could increase the efficacy of verbenone.

MATERIALS AND METHODS

Two randomized complete block, 12-replicate, field trapping experiments (Exp. 1 and 2) were set up on 31 July and 13 August 2002, respectively, near the East Gate of Manning Park BC (49° 19' N, 120° 35' W). Tree protection experiments (Exp. 3A and 4A) with treatments identical to those in the trapping experiments were set up on 5-7 July 2002 in the valley of Whipsaw Creek near Princeton BC (49° 9' N, 120° 41' W), and two additional identical experiments (Exp. 3B and 4B) were set up on 24-27 July 2002 on the 1400 Road south of Prince George BC (53° 21' N, 123° 10' W).

For trapping experiments, 12-unit multiple-funnel traps were deployed at least 15 m apart along logging roads that passed near infested stands. For the tree protection experiments, lodgepole pines with a minimum diameter at breast height (dbh = 1.3 m) of 20 cm were selected at least 25 m apart in rows at least 50 m apart through cut blocks designated for harvest in the fall of 2002.

Treatments (Tables 1, 2) in Exp. 1, 3A and 3B were an unbaited trap or tree (negative control), and an attractive bait alone (positive control) or with MCH, 2-phenyl ethanol, verbenone, or all three together. In Exp. 2, 4A and 4B, the control treatments were the same, but the three disruptants were deployed in all three possible binary blends and the ternary blend. All semiochemicals and release devices were purchased from Phero Tech Inc., Delta BC. The attractive trap bait consisted of the host kairomone myrcene released from a 20 mL polyethylene bottle at 95 mg/24 h, determined at 23 °C, and the aggregation pheromones 82% (-)-*trans*-verbenol and (±)-*exo*-brevicomin respectively released from bubble caps and polyurethane flexlures at 1.2 and 0.3 mg/24 h, determined at 20 °C. The attractive tree bait was identical to the trap bait, but with myrcene deleted (Borden *et al.* 1993). MCH, 2-phenyl ethanol and 80% (-)-verbenone were released from bubble caps at 4.0, 4.2 and 1.8 mg/24 h, determined at 20, 25, and 20 °C, respectively. Devices were hung in the central funnel of traps and affixed to the north face of trees at maximum reach from the ground. The dbh of all baited trees was measured, and varied among experiments (mean ± SE) as follows: 30.9 ± 1.3 cm to 35.1 ± 1.2 cm in Exp. 3A and 4A near Princeton, and 23.0 ± 0.5 cm to 25.3 ± 0.9 cm in Exp. 3B and 4B near Prince George.

Captured beetles in Exp. 1 and 2 were collected on 13 and 26 August, respectively. Beetles were held at ca. -5 °C in plastic bags until sexed and counted.

Tree protection experiments were evaluated on 25-27 September (Exp. 3B and 4B) and 3-4 October (Exp. 3A and 4A). The attack density was counted at eye level in two 20 x 40 cm panels on the east and west faces of baited trees and all trees

Table 1.
Effect of MCH, 2 phenyl ethanol and verbenone alone or in binary or ternary combinations, on catches of mountain pine beetles in attractant-baited multiple-funnel traps. Eastgate Road near Manning Park, B.C., 31 July – 13 August 2002 for Experiment 1 and 13 – 26 August for Experiment 2.

Exp. No.	Treatment[1]	No. reps	Mean number of beetles captured (±SE)[2]	
			Males	Females
1	MPB bait	12	137.8 ± 47.4 a	86.5 ± 38.8 a
	Bait + MCH	12	44.9 ± 8.9 b	24.4 ± 8.2 b
	Bait + 2 PE	12	4.7 ± 1.1 c	4.3 ± 1.2 c
	Bait + V	12	3.5 ± 1.2 cd	2.3 ± 1.5 cd
	Bait + V + MCH + 2PE	12	1.8 ± 0.5 cd	1.1 ± 0.8 d
	Unbaited	12	0.8 ± 0.4 d	0.8 ± 0.3 d
2	MPB bait	12	76.1 ± 29.8 a	41.3 ± 16.0 a
	Bait + MCH + 2PE	10	1.7 ± 0.7 b	1.6 ± 0.7 b
	Bait + V + MCH	12	2.2 ± 1.5 b	1.1 ± 0.6 b
	Bait + V + 2PE	12	0.5 ± 0.2 b	0.2 ± 0.2 b
	Bait + V + MCH + 2PE	12	0.4 ± 0.2 b	0.6 + 0.2 b
	Unbaited	12	0.1 ± 0.1 b	0.3 ± 0.1 b

[1] Treatments as follows: MPB bait = mountain pine beetle bait including *trans*-verbenol, *exo*-brevicomin and myrcene; MCH= 3-methyl-2-cyclohexen-1-one; 2PE= 2-phenyl ethanol; V=verbonene.
[2] Means followed by the same letter are not significantly different, REGW test, $P<0.05$ ANOVA results: Exp. 1., males, F=53.87, df=5,66, $P<0.0001$; Exp. 1, females, F=34.89, df=5,66, $P<0.0001$; Exp. 2, males, F=14.45, df=16,52, $P<0.0001$; Exp. 2, females, F=9.84, df=16,52, $P<0.0001$.

with at least five attacks in the total 0.16 m^2 area (31.25/m^2) were classed as mass-attacked. All surrounding lodgepole pines at least 17.5 cm dbh within 5 m of baited trees were evaluated as unattacked, attacked, or mass-attacked, the latter being determined qualitatively by visual estimation of attack density and copious amounts of frass in bark crevices and around the root collar.

Data for numbers of beetles captured and attack density on baited trees were log-transformed and analyzed by ANOVA and the REGW test (Day and Quinn 1989). Data on proportions of baited and surrounding trees that were mass-attacked were analyzed by chi-square tests for comparison between multiple proportions (Jones 1984). In all cases $\alpha = 0.05$.

RESULTS

In the first trapping experiment (Exp. 1), MCH, 2-phenyl ethanol and verbenone reduced the catches of male and female mountain pine beetles in attractant-baited traps by 67.4%, 71.8% and 96.6%, and 95.1%, and 97.5% and 97.3%, respectively, relative to catches in baited control traps (Table 1). 2-Phenyl ethanol reduced catches to levels no different from those achieved by verbenone (both sexes) or the ternary blend (males only), but only the latter two treatments resulted in catches not significantly different from those in unbaited control traps. In Exp. 2, all binary

Table 2.

Effect of treatment with MCH, 2-phenyl ethanol and verbenone alone, or in binary or ternary combinations, on ranked percentages of pheromone-baited lodgepole pines that were mass-attacked, and on the pooled percentages of all surrounding trees ≥ 17.5 cm dbh within 5 m of the baited tree that were mass-attacked.

Exp. no. (no. reps)	Location	Treatment[1]	Percent baited trees mass-attacked[2]	Surrounding trees	
				N	Percent mass-attacked[2]
3A (25)	Princeton	MPB bait	100.00 a	103	15.5 ab
		Bait + MCH	84.0 b	119	23.5 a
		Bait + 2PE	66.7 b	104	4.8 b
		Bait +V + MCH + 2PE	0.0 c	127	0.0 c
		Unbaited	0.0 c	100	0.0 c
		Bait + V	0.0 c	117	0.0 c
3B(20)	Prince George	MPB bait	95.0 a	69	47.8 ab
		Bait + MCH	80.0 ab	83	31.3 ab
		Bait + 2PE	75.0 abc	62	24.2 b
		Unbaited	40.0 bc	65	49.2 a
		Bait +V + MCH + 2PE	40.0 bc	75	18.7 b
		Bait + V	35.0 c	70	27.1 ab
4A (20)	Princeton	MPB bait	100.0 a	96	26.0 a
		Bait + MCH + 2PE	60.0 b	93	15.1 a
		Bait + V + MCH	5.3 c	87	1.1 b
		Bait + V + 2PE	0.0 c	89	0.0 b
		Unbaited	0.0 c	103	0.0 b
		Bait + V + MCH + 2PE	0.0 c	105	0.0 b
4B (17)	Prince George	MPB bait	94.1 a	52	44.2 a
		Bait + V + MCH + 2PE	47.1 b	47	25.5 ab
		Bait + V + 2PE	35.3 b	61	23.0 ab
		Unbaited	29.4 b	67	17.9 b
		Bait + MCH + 2PE	23.5 b	52	36.5 ab
		Bait + V + MCH	17.6 b	51	25.5 ab

[1] Treatments as in Table 1, Footnote 1, except that myrcene is not present in MPB bait.
[2] Percents within a column and experiment followed the same letter are not significantly different, chi-square test for multiple proportions, $P<0.05$.

combinations and the ternary combination of disruptants reduced catches by more than 96%, and in all cases catches in traps with disruptive treatments were no greater than in unbaited control traps.

In the first tree protection experiment near Princeton (Exp. 3A), all pheromone-baited control trees were mass-attacked (Table 2). MCH and 2-phenyl ethanol alone reduced the proportion of baited trees that were mass-attacked by 16.0% and 33.3%, respectively, but verbenone and the ternary blend completely protected pheromone-baited trees and all trees within 5 m of them from attack. In Exp. 3B near Prince George, only verbenone and the ternary blend significantly reduced the percentage of baited trees that were mass-attacked, and the lowest percentages of surrounding trees that were mass-attacked occurred in the 2-phenyl ethanol and ternary blend treatments.

In the second tree protection experiment near Princeton (Exp. 4A), the binary combination of MCH and 2-phenyl ethanol reduced the percentage of baited trees that were mass-attacked by 40%, but did not cause a reduction in the proportion of surrounding trees that were mass-attacked (Table 2). All treatments containing verbenone reduced attack to zero or to a level not significantly different from zero. In Exp. 4B near Prince George, all treatments significantly and equally reduced the percentage of baited trees that were mass-attacked, but no treatment had a significant effect on attack on surrounding trees.

In all cases except Exp. 3B (Bait + V + MCH + 2 PE), disruptant treatments including verbenone caused a reduction in attack density on mass-attacked trees relative to the MPB bait alone, but in the absence of verbenone, only MCH + 2-phenyl ethanol in Exp. 4B caused a similar reduction (Table 3).

Table 3.

Effect of treatment with MCH, 2-phenyl ethanol and verbenone alone, or in binary or ternary combinations, on ranked densities of attack by the mountain pine beetle on pheromone-baited lodgepole pines.

Exp. no. (no. reps)	Location	Treatment[1]	No. attacked trees	Mean attack density/ m^2 ± SE on attacked trees[2]
3A (25)	Princeton	MPB bait	25	125.5 ± 10.1 a
		Bait + 2PE	16	112.5 ± 13.8 a
		Bait + MCH	23	108.1 ± 7.6 a
		Bait + V + MCH + 2PE	3	6.3 ± 0.0 b
		Bait + V	0	no attack
		Unbaited	0	no attack
3B (20)	Prince George	Bait + MCH	15	105.4 ± 10.5 a
		MPB bait	19	91.4 ± 8.5 ab
		Bait + 2PE	14	77.3 ± 10.4 ab
		Unbaited	10	62.5 ± 13.8 bc
		Bait + V + MCH + 2PE	8	60.9 ± 7.6 bc
		Bait + V	13	52.9 ± 10.2 c
4A (20)	Princeton	MPB bait	20	129.1 ± 8.4 a
		Bait + MCH + 2PE	14	98.7 ± 14.8 a
		Bait + V + MCH	6	25.0 ± 11.9 b
		Bait + V+ 2PE	5	8.8 ± 4.3 b
		Bait + V + MCH + 2PE	6	8.3 ± 3.5 b
		Unbaited	1	no attack
4B (17)	Prince George	MPB bait	15	116.3 ± 9.1 a
		Bait + V + MCH + 2PE	11	64.2 ± 40.1 b
		Bait + V + 2PE	9	58.3 ± 11.3 b
		Unbaited	9	43.8 ± 10.8 b
		Bait + MCH + 2PE	12	40.6 ± 13.9 b
		Bait + V + MCH	7	40.2 ± 14.8 b

[1] Treatments as in Table 1, Footnote 1, except that myrcene was not present in MPB baits.

[2] Percents within a column and experiment followed the same letter are not significantly different, chi-square test for multiple proportions, $P<0.05$.

DISCUSSION

Our results confirm the bioactivity of 2-phenyl ethanol as an antiaggregation pheromone of the mountain pine beetle (Pureswaran *et al.* 2000), and MCH as a repellant synomone (Pureswaran and Borden 2004).

Unlike MCH, 2-phenyl ethanol appeared to have some potential as a pest management tool. MCH caused only marginal reductions in trap catches, and afforded little protection of trees. In contrast, 2-phenyl ethanol caused large reductions in trap catches and greater protection of pheromone-baited and surrounding trees. In Exp. 1, 2, 3A and 3B in the southern part of the province, verbenone was so effective alone that there was no opportunity to observe any potential interaction between MCH, 2-phenyl ethanol and verbenone. However, near Prince George, where beetle pressure was much higher than near Princeton, adding MCH, 2-phenyl ethanol or both together to verbenone did not cause any greater protection of pheromone-baited or surrounding trees than was achieved by verbenone alone. In a similar experiment nonhost volatiles added to verbenone resulted in substantially greater protection of baited and surrounding trees than was found with verbenone alone (Huber and Borden 2001). However, in a small plot experiment the same nonhost volatile blend caused a greater reduction of attack than verbenone alone when added to high-dose verbenone pouches, but not when added to the same low-dose bubble caps used in our experiments (Borden *et al.* 2003).

Our results show that when tested alone, 2-phenyl ethanol was more effective than MCH in reducing catches in attractant-baited traps (Table 1), as well as the percentage of baited and surrounding trees that were mass-attacked (Table 2, Exp. 3A). However, neither MCH nor 2-phenyl ethanol alone reduced the attack density of trees that were mass-attacked (Table 3), and there was no apparent additive or synergistic effect of combining the two compounds or of adding them alone or together to verbenone to protect trees from attack. Therefore, we conclude that neither compound has compelling potential for use as an adjuvant to verbenone in operational attack disruption programs.

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Heteroptera (Hemiptera: Prosorrhyncha) New to Canada. Part 2

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ABSTRACT

The occurrence of an additional six species of true bugs newly recognized in Canada is documented. New US state records are given for two pentatomid species, and a key to the species of *Neottiglossa* Kirby in Canada is included.

INTRODUCTION

In a previous paper (Scudder 2000), 34 species of true bugs newly recognized in Canada was documented. At that time, it was noted that additional species would be published in Part 2, when all determinations had been confirmed.

In the intervening period, additional species have been included in publications by Schuh (2001), Schwartz and Scudder (2001, 2003) and Paiero *et al.* (2004).

I now report an additional six species new to Canada.

Museum abbreviations used in the text are as follows:

APM: Alberta Provincial Museum, Edmonton, AB.

CNC: Canadian National Collection of Insects, Agriculture and Agri-Food Canada, Ottawa, ON.

LM: Lyman Entomological Museum, Macdonald College, McGill University, Ste.-Anne-de-Bellevue, QC.

PFC: Pacific Forestry Centre, Natural Resources Canada, Victoria, BC.

UA: Strickland Museum, University of Alberta, Edmonton, AB.

UBC: Spencer Entomological Museum, Department of Zoology, University of British Columbia, Vancouver, B.C.

SPECIES NEW TO CANADA

Family CYDNIDAE

Amnestus basidentatus Froeschner

QC: 2♂ 2♀, Aylmer, lumière, Boisé décidu., 29.v.1989 (L. LeSage) [CNC].

This species has been swept from grass, and occurs from New York south to Florida and Cuba, and west to Missouri and Texas (Froeschner 1960). A key to separate *A. basidentatus* from the other three species of *Amnestus* Dallas that occur in Canada (Maw *et al.* 2000) is provided by McPherson (1982). *A. basidentatus* has four marginal pegs on each juga, and the male has a characteristic anterior subbasal tooth on the front tibia, giving this segment a notched appearance on its inner surface. The ventral subapical spine on the hind femur of the male is also shorter than the width of the femur, while in the female, the last abdominal sternum lacks a flattened glabrous area.

Family MIRIDAE

Pinophylus carneolus (Knight)

SK: 1♂, Nipawin, Jack pine, 7.vi.1968 (FIS 605) [CNC].

The genus *Pinophylus* Schwartz & Schuh was described by Schwartz and Schuh (1999) with three contained species, one of which *P. rolfsi* (Knight), was reported from Alberta, British Columbia and Yukon, and south to Oregon and Colorado. *Pinophylus carneolus* was reported from District of Columbia, Maryland, North Carolina, Pennsylvania, Virginia, West

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Virginia and Wisconsin, and like *P. rolfsi* is strongly sexually dimorphic. *P. carneolus* is distinguished from *P. rolfsi* by the slightly reddish brown coloration, and the form of the male genitalia in which the vesica is more strongly curved than in *P. rolfsi*. *P. carneolus* is reported to breed on Virginia pine, *Pinus virginiana* Mill. in the United States (Knight 1927; Schwartz and Schuh 1999).

Family PENTATOMIDAE

Neottiglossa sulcifrons Stål

SK: 1♀, Indian Head, Aspen Grove, 3.viii. 1939 (C.R. Douglas) [CNC].

Four species of the genus *Neottiglossa* Kirby are now known from Canada. Two of these, *N. trilineata* (Kirby) and *N. undata* (Say), occur across Canada from Yukon to Newfoundland, while *N. tumidifrons* Downes is confined to British Columbia in Canada (Maw *et al.* 2000). The following key modified from that in Rider (1989), will separate the species of *Neottiglossa* now known from Canada.

1. Coxae pale yellow; evaporative surface on pterothorax ventrally pale yellow to brown-grey with contrasting black punctures 2

-Coxae fuscous to black; evaporative surfaces on pterothorax ventrally black with concolorous punctures 3

2. Dorsal surface of head and propleura mostly black with concolorous punctures ..... *strilineata* (Kirby)

-Dorsal surface of head and propleura with large areas pale yellow to brown with black puncture..... *sundata* (Say)

3. Trochanters dark fuscous; scutellum lacking pale median line ..... *sulcifrons* Stål

-Trochanters pale; scutellum usually with pale median line. *tumidifrons* Downes

N. sulcifrons is usually collected in grassy habitats (McPherson 1982) and is recorded through much of the eastern and central Untied States, south to Georgia, Texas, New Mexico and Arizona. Froeschner (1988) and Rider (1989) summarize the records for the US states. To these can be added the following new state records.

COLORADO: 1♀, Boulder, Flagstaff Cn., 5800' (1705 m), 8.viii. 1961 (J.R. Stainer); 1♂ 1♀, *id*, 11.vi.1961; 1♀, Boulder, 12.vi.1961 (B.H. Poole); 2♀, Mt. Evans, 9800' (2987 m), Doolittle Ranch, 10.viii. 1961 (B.H. Poole); 1♂, Nederland, Science Lodge, 9500' (2896 m), 1.vii.1961 (J.R. Stainer); 1♂, *id*, 6.vii.1961; 1♀, *id*, 9000' (2743 m), 29.vii.1961; 3♀, Nederland, Caribou, 8700' (2652 m), 7.viii. 1961 (J.R. Stainer). OKLAHOMA: 1♀, Texoma Lk., 15.vii.1954 (J.G. Chillcott). SOUTH CAROLINA: 1♀, Aiken, 24.viii. 1957 (W.R. Richards); 1♀, Montmorenci, 23.vi.1957 (W.R.M. Mason) [CNC].

Little is known about the life history and habits of *N. trilineata* (McPherson 1982). It is recorded from Alaska (Scudder 1997) (1♂, Fairbanks, 16.vi.1952 (J.H. Hartley) [CNC]) and south in the United States to California, Colorado, Wyoming and Nebraska (Froeschner 1988; Rider 1989).

N. tumidifrons is a Cordilleran species recorded from California, Oregon and Washington, in addition to British Columbia. It occurs in grassy habitats, and in British Columbia is confined to southeastern Vancouver Island, and the dry southern interior of the province. *N. undata* is much more widely distributed, occurring in grassy habitats from Alaska to California in the west, and from Newfoundland south to North Carolina in the east (McPherson 1982).

The fifth species in North America, *N. cavifrons* Stål, occurs from Virginia south to Georgia and South Carolina (New Record: 1♀, SC, Montmorenci, 23.vi.1957 (W.R.M. Mason) [CNC]) in the east. There are records from Arizona, California, Oregon, Texas and Utah in the west (Froeschner 1988; Rider 1989) , but the early reports of this species from British Columbia (Stoner 1926; Downes 1927) are in error, as these records refer to *N. tumidifrons* (Downes 1935).

Trichopepla grossa Van Duzee

BC: 1♂, Osoyoos IRI, 'Brights Winery', *Purshia* assoc., BGxh1 AN, Pitfall trap V2-4, 2.vi.-7.vii.1994 (G.G.E. Scudder) [UBC]; 1♀, Fairview, White Lake, Big

sage assoc., BGxh1 SWm, 7.vii.1996 (G.G.E. Scudder) [UBC].

T. grossa is a Cordilleran species, previously recorded from California, Colorado, Idaho, Oregon and South Dakota (McDonald 1976). Four other species of *Trichopepla* Stål are recorded from Canada (Maw *et al.* 2000), and *T. grossa* is most similar to *T. aurora* Van Duzee, which in Canada is also confined to British Columbia. McDonald (1976) gives a key to separate the species. *T. grossa* has the abdominal connexiva rather uniform pale brown or yellowish marginally, the scutellum has a pale yellow tip, and the base of the pronotum and coria are concolorous with rest of the dorsal surface. In contrast, *T. aurora* has an alternating pattern of black and pale brown on the abdominal connexiva, the tip of the scutellum is concolourous and not pale, while the base of the pronotum and the coria are usually clearly roseus.

Family THYREOCORIDAE

Galgupha ovalis Hussey

In Canada, first reported from Alberta and British Columbia in Maw *et al.* (2000), without data. Now also known from Saskatchewan. Specimens examined: 57♂ 82♀.

AB: Elkwater, 15.vi.1955 (George E. Ball) [UA]; Gull Lake, 8.vi.1929, 14.vi.1929, 22.vi.1929 (E.H. Strickland) [UA], previously determined as *G. nitiduloides* (Wolff); Medicine Hat [LM]; CFB Suffield, NWA, 26.v.1994, 16-28.vi.1994, 16-29.vi.1994, 28.vi.1994, 28.vii.-16.viii. 1994, 16.vi.1995, 29.vi.1995, 31.vii.1995 (A.T. Finnamore) [APM]. BC: Chopaka, 12.v.1983 (S.G. Cannings); Chopaka, SATH habitat, BGxh1 SN pitfall trap CH6-2, 23.vi.-18.vii.1996 (J. Jarrett); Fairview, White L., Big sage assoc., BGxh1 SWm, 7.vii.1996 (G.G.E. Scudder) [UBC]; Enderby, 22.viii. 1920 (W. Downes) [UBC], as *G. atra* A. & S. in Parshley (1921), Downes (1927) and Walley (1929); Keremeos Creek, 2000' (607 m) sagebrush flat, fall trap, 9.vii.1982, 16.vii.1982, 23.vii.1982 (H. Kirk) [UBC]; near Oliver, 22-23.v.1958 (G.E. Ball) [UA]; Oliver, 29.v.1924 (P.N. Vroom) [PFC]; Oliver, 26.v.1945 (D. Blair) [UBC]; Oliver, IRI, 'Water tower', *Purshia* assoc., BGxh1 AN, pitfall trap U2-4, 1.vi.-7.vii.1994 (G.G.E. Scudder) [UBC]; Oliver, McIntyre Cr., 3000' (915 m), 29.v.1958 (H. & A. Howden) [CNC]; Oliver, 5 mi. N., 21.v.1958 (H. & A. Howden) [CNC]; Oliver, 8 mi. N., 18.v.1958, 19.v.1958 (H. & A. Howden) [CNC]; Osoyoos, 21.v.1924 (K.F. Auden) [PFC]; Osoyoos, 19.v.1958 (H. & A. Howden) [CNC]; Osoyoos, 49°03'N 119°31'W [Desert Centre], BGxh1 8PD/2AN:P, pitfall trap, 19.vii.-17.viii. 1996, 23.vi.-28.vii.1997, 27.vii.-17.viii.1997, 17.viii.-21.ix.1997 (J. Jarrett) [UBC]; Osoyoos, East Bench, 22.viii. 1995 (G.G.E. Scudder) [UBC]; Osoyoos, Haynes Ecol. Res., 11.v.1982, 15.v.1985 (S.G. Cannings) [UBC]; Osoyoos, Haynes Ecol. Res., BGxh1 AN, recovery after fire, pitfall trap, 9.vii.-7.viii. 1994 (G.G.E. Scudder) [UBC]; Osoyoos, IRI, Inkaneep, *Purshia* Assoc., BGxh1 AN, pitfall trap T5-5, 6.vii.-9.viii.1995 (G.G.E. Scudder) [UBC]; Penticton, 17.v.1985 (R.J. Cannings) [UBC]; Quesnel, 11.vii.1948 (G.J. Spencer) [CNC]; Rock Creek, 7.vi.1959 (L.A. Kelton) [CNC]; Ross Lake, Okanagan Falls, 5.vi.1959 (R.E. Leech) [CNC]; Vaseux L., 5.vii.1981 (S.G. Cannings) [UBC]; Vaseux L., 2000' (607 m), 13.vi.1983 (R.J. Cannings) [UBC]; Vaseux L., 2-3 mi. E., 24.v.1958 (G.E. Ball) [UA]; White L., Okanagan Falls, ex. *Plantago*, 6.vii.1985 (R.J. Cannings) [UBC]. SK: Cypress Hills Prov. Pk., Rte. 221, 27.4 km. E. Pt. Walsh prairie, 18.v.1976 (Danny Shpeley, George E. Ball) [UA]; Regina, 2.vi.1943 (P. Larkin) [CNC].

G. ovalis has been reported to occur on *Pycnanthemum* and *Vernonia*, and in the United States ranges from Massachusetts west to Montana, and south to Florida, Arizona, Texas and Guatemala (McPherson 1982). McPherson (1982) provides a key to separate *G. ovalis* from the other three species reported from Canada (Maw *et al.* 2000). In *G. ovalis*, the metapleura laterally are impunctate, the corium has a distinct ridge inside the costal

groove, the scutellum is gradually declivent posteriorly, and the posterior border of the pygophore in the male, when viewed from below is weakly concave, and the dorsal rim lacks numerous long setae posteriorly.

Family TINGIDAE

Stephanitis takeyai Drake & Maa

BC: 17♂ 33♀, Richmond, ex. *Pieris japonica* (Thunb.) D. Don, 31 September 2001 (R. Costello) [CNC, UBC].

S. takeyai is an alien species in North America, first reported on *Pieris japonica* (Japanese andromeda) at Greenwich, Connecticut in 1946 (Bailey 1950). Subsequently, it has been reported in several other eastern states (Dunbar 1974; Wheeler 1977) where it also occurs in nursery and landscape plantings on *Lindera bezoin* (L.) Blume (spicebush) and *Sassafras albidum* (Nutt.) Nees (Wheeler 1977).

The occurrence of this tingid, commonly called the Andromeda Lace Bug, in British Columbia represents the first record of this alien species in Western North America. It is likely the result of a separate introduction via nursery stock.

S. takeyai can be separated from *S. rhododendri* Horvath, another alien species in Canada that has separate introductions in both the east and west in Canada, by its more inflated and higher pronotal hood, its shorter lateral carinae on the pronotum, and by the much darker markings on the hood and hemelytra. The paranota are almost vertical in *S. takeyai*, whereas in *S. rhododendri* they are more flared (Bailey 1950).

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Lestes disjunctus Selys and *L. forcipatus* Rambur (Odonata: Lestidae): Some Solutions for Identification

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ABSTRACT

Five species of the damselfly genus *Lestes* live in British Columbia, Canada, and of these, *Lestes forcipatus* Rambur and *L. disjunctus* Selys are the most similar and most difficult to separate morphologically. Females can be readily distinguished by the size of the ovipositor, but males are difficult to separate. In British Columbia, *L. disjunctus* is the more common, widespread and familiar species. Before 1998, *L. forcipatus* specimens were mistaken for those of *L. disjunctus* because the former is primarily an eastern North American species and because most *Lestes* species are usually identified using male characters. With the discovery that *L. forcipatus* is part of the western fauna, an evaluation of the relative status of the two species in British Columbia is necessary. The best method for separating the two species uses the length of the anterior lamina (part of the secondary genitalia) as a unique character or as part of ratios using other measurements. In addition, in at least western North America, *L. forcipatus* males are more pruinescent than those of *L. disjunctus*, especially on the thorax. Identification using the pruinescence pattern was tested in the field and is recommended as a simple and accurate method for western North America. Soaking Odonata specimens in acetone, a common technique used to preserve colours, damages surface pruinescence and should not be used to preserve mature, pruinescent adults, including those of *Lestes* species. To identify *L disjunctus* and *L. forcipatus* males treated in acetone, it may be necessary to calculate ratios based on various character measurements. Future research should investigate spatial and temporal differences between the species, as well as modes of interspecific communication.

Key Words: Odonata, *Lestes forcipatus, Lestes disjunctus*, identification, British Columbia, pruinescence, acetone, anterior lamina.

INTRODUCTION

Five species of the damselfly genus *Lestes* (Odonata: Zygoptera: Lestidae) occur in British Columbia (BC), Canada: *L. congener* Hagen (Spotted Spreadwing), *L. disjunctus* Selys (Northern Spreadwing), *L. dryas* Kirby (Emerald Spreadwing), *L. forcipatus* Rambur (Sweetflag Spreadwing), and *L. unguiculatus* Hagen (Lyre-tipped Spreadwing). *L. disjunctus* is the most common, widespread and familiar *Lestes* species in the province, and one of the most abundant odonates in Canada, ranging as far north as the Arctic treeline (Cannings 2002). It inhabits many types of standing water habitats with abundant aquatic vegetation and, in southern BC, adults fly from mid-June to mid-October (Cannings 2002).

L. forcipatus is generally much less common than *L. disjunctus,* although it is as abundant in some cold fen habitats, and both species often occur at the same site. *L. forcipatus* does not range as far north as *L. disjunctus* and, although not known from much of BC's north, it has been collected in the southeastern Yukon. In the western Canadian Cordillera, it is most common in sedge fens (Cannings 2002). Walker

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(1953) described *L. forcipatus* habitat in Ontario as "ponds, both temporary and permanent, marshy lakes, and slow, weedy streams". In BC *L. forcipatus* has been collected from mid-June to mid-September (Cannings 2002).

L. forcipatus was not reported in BC until 1998, when it was first collected in the Rocky Mountain Trench north of Golden and subsequently found in many other localities in the southeastern part of the province. However, it probably has long been a resident of the province; it was long overlooked because of its close resemblance to *L. disjunctus* (Ramsay and Cannings 2000). Before 1998, *L. forcipatus* was not known west of Saskatchewan (Walker 1953, Westfall and May 1996), and had just recently been found in Washington State, the first record west of Montana (Ramsay and Cannings 2000). The species is now known from seven counties in that state and one in Idaho (Paulson 2004). By 1999 *L. forcipatus* had been discovered at several other BC locations farther south and west, and by 2000 had been collected on Vancouver Island. Some of our old museum specimens of *L. disjunctus* from many regions of the province have been re-identified as *L. forcipatus,* indicating that museum collections across western Canada probably contain many misidentified specimens.

Males of *L. disjunctus* and *L. forcipatus* are difficult to separate, although numerous characters have been employed in identification (Walker 1953, Westfall and May 1996, Catling 2002, Donnelly 2003). The usual method of distinguishing the two species and confirming their presence at a location is through identification of the females. In *L. forcipatus* females the ovipositor valves reach the tips of the cerci; in *L. disjunctus* they do not (Walker 1953, Cannings 2002) (Fig. 1).

Lestes species are usually brown, black, metallic green or bronze above and mostly pale below; especially in males, the head, thorax, base and tip of abdomen become pruinescent bluish white with age. Pruinescence (pruinosity) is a waxy substance produced by the hypodermis in many groups of Odonata and excreted on the cuticular surface through porous canals (Gorb 1994). Pruinescence is implicated in thermal regulation in dragonflies (Garrison 1976, Paulson 1983) and is thought to play a role in species recognition and intraspecific communication -- indeed, the patterns of pruinescence in males may be a result of sexual selection (Jacobs 1955, Corbet 1999). Therefore, pruinescence patterns might offer good species identification characters, especially in males.

The object of this project was to find novel and definitive distinguishing characteristics between males of *L. disjunctus* and *L. forcipatus*, building on the studies of workers in eastern North America. Thus, we hope (a) to distinguish males in the absence of associated females; (b) to identify, with relative ease, the species in the field, (c) to correct any misidentifications of specimens in BC museum collections; and (d) to establish accurate distributions for both species in BC. The first part of the present work measures certain structures of the male genitalia to find the best features to separate the species. The second part quantifies the degree of pruinescence of adult males of each species.

MATERIALS AND METHODS

Specimens. We measured 50 male *L. disjunctus* and 45 male *L. forcipatus* specimens from localities in BC and Alberta (two *L. disjunctus* only from Alberta) and from Washington and Maine in the United States. Eighty-four of the specimens were from the Royal British Columbia Museum (RBCM), Victoria; the others were borrowed from the Spencer Entomological Museum, UBC, Vancouver, and the Slater Museum of Natural History, Tacoma, WA. A list of the specimens and their collection data is on file at the RBCM and is available on request. Most specimens were *in copula* or in tandem, except for three *L. forcipatus* and one *L. disjunctus*; thus, the

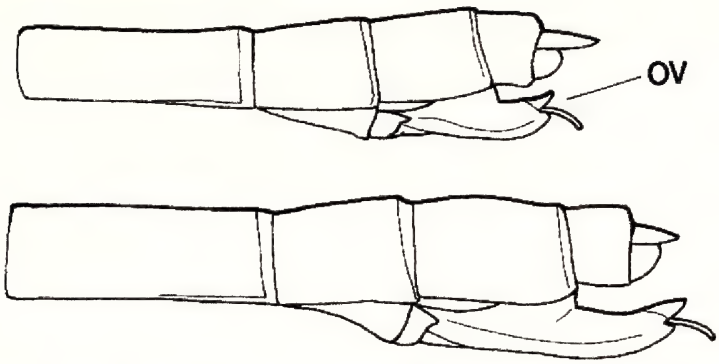

Figure 1. Lateral view of apex of female abdomen. Top, *Lestes disjunctus*; Bottom, *L. forcipatus*. OV = ovipositor.

identities of almost all males were confirmed using the associated females.

Measurements. During examination, each specimen was held by the base of the wings using a small padded alligator clamp soldered to a #7 insect pin. The pin was inserted into a cork mount, and the specimen held in a standardized measuring position. Specimens were examined at 100x magnification and measurements were made to 0.01 mm.

Thirteen characters were measured; terminology follows Westfall and May (1996) and Donnelly (2003).

Cercus (Fig. 2):

1. Distance from base of apical tooth to base of basal tooth (AB).

2. Distance from apex of cercus to base of apical tooth (AC).

3. Distance from swelling at medial base of cercus to base of basal tooth (BB) (not figured).

Secondary genitalia (abdominal segment 2) (Fig. 3):

4. Length of the anterior lamina (anterior hamule) (AL). Walker (1952, 1953) did not explain how to measure the lamina, but Catling (2002) and Donnelly (2003) prefer to measure the ventral length of the hamule from where it appears from above sternite 1. He notes, however, that specimens show different degrees of bending in abdominal segments 1 and 2 and thus there is no good reference for the hamule base. We measured the blade of the lamina only.

5. Length of membranous shield of sperm vesicle (MS) (penis vesicle of Catling (2002) and Donnelly (2003)).

6. Length of penis shaft (PS).

7. Length of sperm vesicle (SV).

Apex of abdominal segment 10 (Fig. 4):

8. Height of apical hood (HT). This structure is a triangular projection on the dorsal apex of abdominal segment 10. The apex of the abdomen was viewed end-on.

9. Width of base of apical hood (HL).

10. Width of the abdomen (WA). The greatest width of the abdomen measured when the apex of the abdomen was viewed end-on.

Other:

11. Length of abdominal segment 2 (S2). Measured in lateral view.

12. Length of abdominal segment 3 (S3). Measured in lateral view.

13. Width of head (HD). The distance between the extreme lateral edges of the eyes, measured dorsally.

We analysed the difference between species for each character measured using a z-test after checking for uniformity of variance, using the MS Excel Data Analysis Tool (Stinson and Dodge 2004).

Pruinescence.

Pterothorax (Fig. 5). We compared the extent of pruinescence on the head, pterothorax (fused mesothorax and metathorax), and abdominal segments 1 to 10 between males of the two species. Pterothoracic pruinescence was divided into several value categories, as follows: absent = 0, low lateral (below interpleural suture) = 1, mid lateral (below midline of mese-

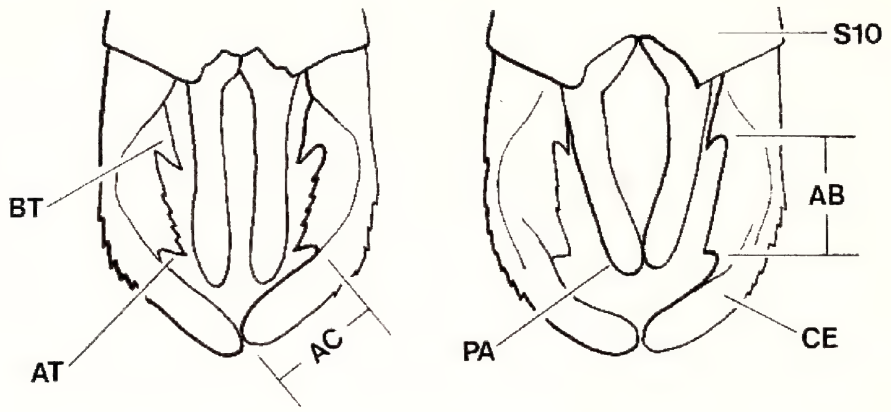

Figure 2. Dorsal view of male primary genitalia. Left, *Lestes disjunctus*; Right, *L. forcipatus*. AB = distance between base of apical tooth and base of basal tooth of cercus, AC = distance between base of apical tooth and apex of cercus, AT = apical tooth of cercus, BT = basal tooth

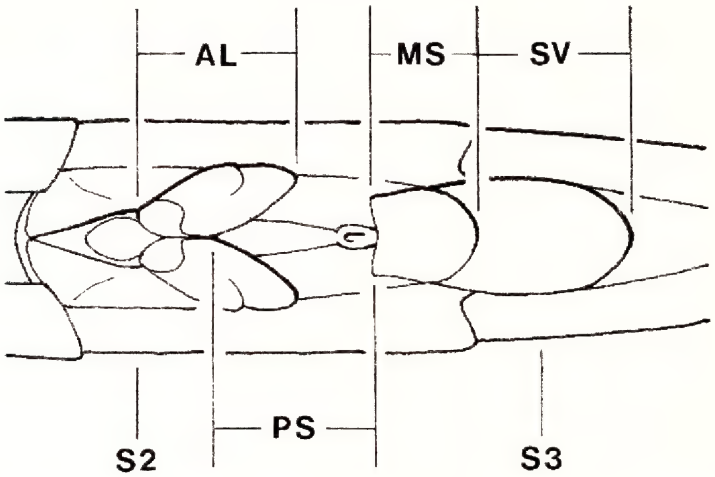

Figure 3. Ventral view of male secondary genitalia. AL = length of blade of anterior lamina, MS = length of membranous shield of sperm vesicle, PS = length of penis shaft, SV = length of sperm vesicle, S2 = abdominal segment 2, S3 = abdominal segment 3.

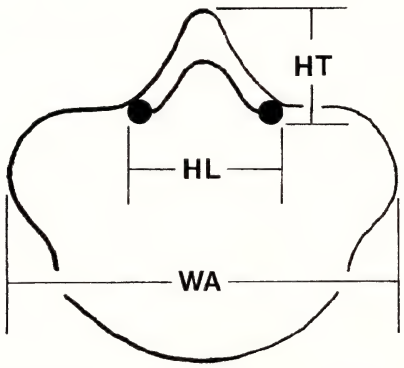

Figure 4. Diagrammatic apical view of abdominal segment 10 of male *Lestes disjunctus*. HT = height of apical hood, HL = width of base of apical hood, WA = width of abdomen.

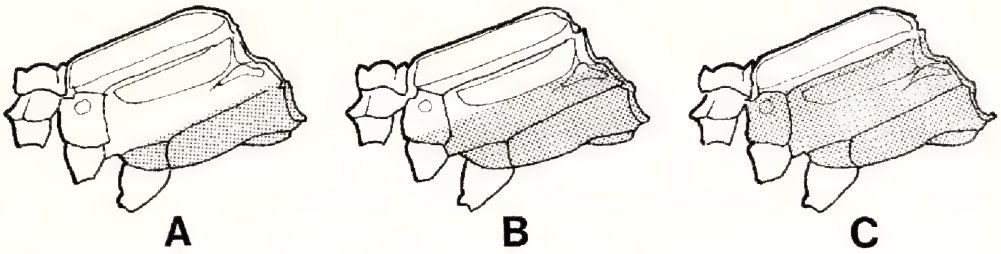

Figure 5. Lateral view of thorax of *Lestes disjunctus* male. Stippling represents coverage of pruinescence: A, low lateral; B, mid lateral; C, complete lateral.

pimeron)= 2, complete lateral (below mesepisternal stripe) = 3, lateral+dorsal (complete lateral plus mesepisternal stripe and dorsal midline) = 4, complete thorax (ventral + lateral + dorsal) = 5. Because specimens had been treated in acetone and the pruinescence patterns were thus damaged, both sides of the pterothorax were compared, and the more pruinescent side recorded.

Abdominal segment 2 (Fig. 6). We examined segment 2 for presence or absence of a rectangular patch free of pruinescence and covering about the apical one-third of the tergite. If the patch was present, we assigned a value of 1; if absent, a value of 0.

RESULTS

Measurements. Nine measurements failed to show a significant difference between the species: AC, BB, HT, HD, MS, PS, S3, SV and WA.

Table 1 summarizes the seven measurements that we consider important to this study; these include the mean, standard deviation, range and significance values for z-tests. In Table 1 the z-test values for the HD, S3, and WA are not significant. However, tests of the same measurements in character ratios show significant differences between the species.

Ratios of character measurements are often useful in preventing individual size variation from obscuring the value of a character when comparing species variation. Analysis showed that several character ratios calculated were not useful in separating the two species: AB/AC, AB/AL, AC/AL, AC/HD, BB/HD, HL/AL, HT/HD, HT/HL, HT/WA, SV/AL, SV/S2, MS/AL, MS/HD, MS/S2, PS/HD, S3/HD, SV/HD, WA/HD. The significant character ratios for both *L. forcipatus* and *L. disjunctus* are summarized in Table 1.

Pruinescence. The head and abdominal segment 1 were pruinescent in all specimens; the pruinescence on segments 3-10 was not significantly different. All comparisons were inconclusive except for those of the pterothorax and abdominal segment 2.

Pterothorax (Table 2). In all specimens of both species, the pterothorax was pruinescent. In *L. forcipatus*, it was completely pruinose (covered ventrally, laterally and dorsally) 72.5% (n = 40) of the time; *L. disjunctus* was never completely pruinescent, and never covered dorsally. *L. forcipatus* was covered completely laterally and dorsally in 20% of specimens but never showed only low lateral or mid lateral pruinescence. Of the 40 specimens measured, three (7.5%) had only the lateral area completely covered. *L. disjunctus* was completely covered laterally 60.0% (n = 30), mid laterally 30%, and low laterally 10% of the time.

Abdominal segment 2. Segment 2 in all *L. forcipatus* specimens had a distinct dorsal bare patch. In *L. disjunctus* an indistinct, different sort of patch was present 23.1% (n = 26) of the time. It was both asymmetrical and lightly pruinescent. The average ± SD patch size (n = 28) in *L. forcipatus* was 0.67 ± 0.13 mm long, by 0.50 ± 0.11 mm wide.

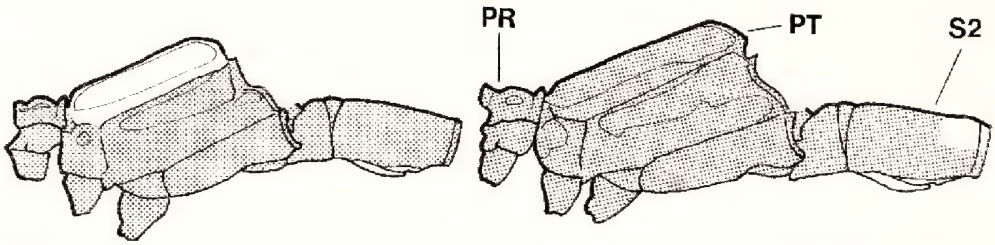

Figure 6. Lateral view of thorax and abdominal segments 1 and 2 of fully pruinescent males. Left, *Lestes disjunctus;* Right, *L. forcipatus*. PR = prothorax, PT = pterothorax, S2 = abdominal segment 2.

Table 1.

Summary statistics of measurements and ratios made for *Lestes forcipatus* and *L. disjunctus*. Two-tailed z-tests were used to compare characters between species. Distances measured are as follows: AB = apical tooth of cercus to basal tooth of cercus, AL = length of blade of anterior hamule, HD = width of head, HL = width of base of apical hood, S2 = lateral length of abdominal segment 2, S3 = lateral length of abdominal segment 3, WA = width of abdomen.

Character	*L. forcipatus*				*L. disjunctus*				z-test results	
Distance	**Mean**	**SD**	**Range**	**n**	**Mean**	**SD**	**Range**	**n**	**z**	**P (Z<=z)**
AB	0.51	0.04	0.47 - 0.60	42	0.45	0.05	0.33 - 0.53	46	6.10	0.00
AL	0.91	0.09	0.67 - 1.20	44	0.72	0.06	0.60 - 1.00	50	11.37	0.00
HD	4.73	0.16	4.33 - 5.00	39	4.77	0.19	4.33 - 5.07	42	-0.89	0.37
HL	0.53	0.06	0.40 - 0.67	41	0.44	0.05	0.33 - 0.53	45	7.42	0.00
S2	2.57	0.16	2.27 - 2.87	44	2.40	0.13	2.13 - 2.67	50	5.47	0.00
S3	4.32	0.24	3.87 - 4.87	44	4.34	0.26	3.93 - 5.00	50	-0.41	0.68
WA	1.27	0.09	1.13 - 1.53	41	1.27	0.10	1.00 - 1.40	45	0.16	0.87
Ratio	**Mean**	**SD**	**Range**	**n**	**Mean**	**SD**	**Range**	**n**	**z**	**P (Z<=z)**
AB/HD	0.11	0.01	0.10 - 0.13	37	0.09	0.01	0.07 - 0.11	40	6.65	0.00
AL/HD	0.19	0.02	0.14 - 0.24	39	0.15	0.01	0.14 - 0.20	42	11.99	0.00
AL/S2	0.35	0.03	0.27 - 0.42	44	0.30	0.03	0.25 - 0.39	49	8.27	0.00
AL/S3	0.21	0.02	0.14 - 0.26	44	0.17	0.01	0.14 - 0.22	49	11.08	0.00
HL/HD	0.11	0.01	0.08 - 0.14	36	0.09	0.01	0.07 - 0.11	38	7.50	0.00
HL/WA	0.42	0.04	0.30 - 0.50	41	0.35	0.04	0.28 - 0.47	45	7.53	0.00
S2/HD	0.55	0.03	0.48 - 0.59	39	0.51	0.02	0. 47 - 0.54	42	7.74	0.00
S2/S3	0.60	0.03	0.52 - 0.64	44	0.55	0.02	0.50 - 0.60	49	8.57	0.00

DISCUSSION

Measurements. The search for diagnostic characters to differentiate *L. forcipatus* males from those of *L. disjunctus* is not a new one. According to Donnelly (2003), the earliest paper that demonstrates a distinction between the two species is by Garman (1917), who illustrated the longer ovipositor in *L. forcipatus.* Montgomery (1941) noted the widespread confusion between the species and cited four diag-

Table 2.
Percentage of specimens of *Lestes forcipatus* and *L. disjunctus* displaying selected patterns of pruinescence on the pterothorax. Figs. 5 and 6 illustrate the patterns.

Pruinosity pattern	*L. forcipatus* % (n=40)	*L. disjunctus* % (n=30)
Lateral-ventral-dorsal	72.5	0
Dorso-lateral	20	0
Complete lateral	7.5	60
Mid lateral	0	30
Low lateral	0	10
Absent	0	0

nostic differences: (1) distance between teeth of cercus; (2) width of apical hood of abdominal segment 10; (3) width of base of membranous shield of sperm vesicle (penis vesicle), and (4) shape of penis (see Donnelly 2003). When Walker (1952) reviewed Montgomery's findings, he rejected the shape of the penis, but retained the other three characters. Walker (1952) added the relative lengths of abdominal segments 2 and 3 and the length of the anterior lamina (see Donnelly 2003). Westfall and May (1996) also base their separation of the species on the relative lengths of abdominal segments 2 and 3, but added the distance between the tip of the basal tooth and the swelling near its base, the shape of the membranous shield of the sperm vesicle and the relative size of the cercal teeth.

Donnelly's (2003) findings are different again. He stressed the use of the anterior lamina length, the distance between the apical and basal teeth on the cercus, the shape of the paraproct and the apical hood width on segment 10. He preferred not to use the membranous shield, the relative lengths of abdominal segments 2 and 3, and the distance from the basal swelling of the cercus to the tip of the basal tooth. Catling's (2002) useful study of Ontario material concluded that the best characters were the relative heights of the apical and basal teeth of the cercus and the relative extent of pale and dark pigment (not pruinescence) on the thorax.

Our findings support the conclusion that it is best to use a combination of characters for identification. In western North America, at least, both morphology and the pattern of pruinescence should be considered. A short review of useful characters and character ratios follows:

1. Anterior lamina (AL). Rather than measuring the whole length of the lamina (including the stalk), we measured the expanded apical blade-like part only. The lamina in *L. forcipatus* is longer (mean = 0.91 mm) than that of *L. disjunctus* (mean = 0.72 mm). The ranges of the lengths of the AL overlap in the two species, but the length in *L. disjunctus* does not exceed 1 mm, while that of *L. forcipatus* reaches 1.20 mm. We found the lamina to be significantly different in three character ratios – those using the head width, the length of segment 2 and the length of segment 3.

2. Base of apical tooth to base of basal tooth (AB). AB is a good identification character as a simple measurement or as a ratio with head width (Table 1). The distance between the teeth is longer in *L. forcipatus* than in *L. disjunctus*; this result is supported by Donnelly (2003). Although there is some overlap in the measurements of the two species (*L. disjunctus,* 0.33 – 0.53 mm; *L. forcipatus,*0.47 – 0.60 mm), the character is useful when used in conjunction with others.

3. Width of the apical hood (HL)

The ranges of apical hood widths overlapped in the two species -- *L. disjunctus* (0.33 – 0.53 mm) and *L. forcipatus* (0.40 – 0.67 mm). The HL is generally greater in

L. forcipatus, which gives the apical hood the wide, low appearance (as opposed to the pinched shape in *L. disjunctus*) that is often used to distinguish the species (Donnelly, 2003, Lam 2004). Based on our data, this is a generalization and is not reliable for differentiating the species. The HL is useful when used in ratios using the head and abdomen.

4. Width of the head (HD). There was no significant difference between the species in the width of the head. We used the measurement to calculate ratios.

5. Lateral lengths of abdominal segments 2 and 3. There was a significant difference between the length of segment 2 in both species; however, the ranges overlapped considerably. Segment 3 was not different between species but the relative lengths of segments 2 and 3 were significant.

6. Width of the abdomen (WA). This measurement is significant only when used in a ratio with measurements of the apical hood. Comparing species using this character is difficult as the ranges overlap greatly.

Pruinescence. The literature from eastern North America, where *L. forcipatus* has been studied for decades, does not mention pruinescence as a basis for separating *L. disjunctus* and *L. forcipatus* (Walker 1952, 1953, Westfall and May 1996, Catling 2002, Donnelly 2003, Lam 2004). In that region, pruinescence patterns are apparently different from those in northwestern North America and are of little use in species identification. On the other hand, as was originally noted in Washington State by Dennis Paulson, (D.R. Paulson, Slater Museum, University of Puget Sound, Tacoma; pers. comm.), in far western North America, pruinescence in mature individuals seems a good character for separating the species. It has the advantage of being easy to use in the field without even having to capture the specimen. Further study of these patterns over the whole range of the two species is required.

Maturity is accompanied by pruinescence on abdominal segments 2, 8, 9, and 10, and to a lesser degree on abdominal segments 3, 6, and 7. Patterns on abdominal segments other than segment 2 are not useful in identification because they are almost identical in shape, intensity and frequency of occurrence in both species. Abdominal segment 2 however, is reliable in differentiating *L. disjunctus* and *L. forcipatus* (Table 2.). Although 23% of *L. disjunctus* appear to have a clear patch at the apex of this segment it has, upon closer inspection, not a clearly defined rectangular shape but an asymmetrical shape with some pruinscence throughout. There was little individual variation in the position of pruinescence in either *L. forcipatus* or *L. disjunctus*.

Conclusions. Even with careful analysis of each character, a specimen lacking pruinescence is difficult to identify. As a general rule, a specimen with longer or wider measurements than the average *L. disjunctus* specimen should be regarded as a potential *L. forcipatus*. The most worthwhile characters to choose for identification are the AB (the distance between the base of the apical tooth and the base of the basal tooth of the circus), AL (the length of the blade of the anterior lamina), HL (the basal width of the apical hood), and S2 (the lateral length of abdominal segment 2). In each, the mean distance is higher in *L. forcipatus* and, although ranges overlap considerably, the range exceeds that of *L. disjunctus*.

The most useful ratios are the above measurements divided by the head width (AB/HD, AL/HD and HL/HD). In AB/HD the ranges of the two species overlap minimally compared to those of the other significant ratios. In the remaining two ratios the range of *L. forcipatus* far exceeds that of *L. disjunctus*.

In our study, any specimen with pruinescence on the dorsum of the pterothorax (mesepisternal stripe plus midline) is *L. forcipatus,* and the species showed this trait in over 90% of the specimens examined. *L. forcipatus* never had only low lateral or mid lateral pruinescence, a common pattern in *L. disjunctus,* and showed complete lateral coverage (without any dorsal

pruinescence) only 7.5% of the time, compared to 60% of *L. disjunctus* specimens. Any specimen with a strongly differentiated, symmetrical, pruinescent-free patch apically on the dorsum of abdominal segment 2 was *L. forcipatus*. The segment in *L. disjunctus* was usually completely pruinescent; about a quarter of the time it was marked with an irregular, lightly pruinescent patch.

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Dennis Paulson (Slater Museum of Natural History, Tacoma), Karen Needham and Rex Kenner (Spencer Entomological Museum, UBC, Vancouver) loaned specimens. Dennis Paulson gave useful advice concerning pruinescence patterns. Richard Ring read an early draft of the manuscript.

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SCIENTIFIC NOTE

A ground-based pheromone trap for monitoring *Agriotes lineatus* and *A. obscurus* (Coleoptera: Elateridae)

R.S. VERNON[1]

The dusky click beetle, *Agriotes obscurus* (L.) and the lined click beetle, *A. lineatus* (L.) were introduced to British Columbia (BC) from Europe about a century ago, and are now serious pests of several crops (e.g. corn, cereals, potatoes) in the lower Fraser Valley and Vancouver Island (Wilkinson 1963). Prior to 1999, delimitation surveys of these species in North America were based on: inspections of click beetles in existing private or national collections (Eidt 1953; Vernon and Päts 1997); habitat searches (Brown 1950); or click beetles taken in strategic arrays of pitfall traps (Vernon and Päts 1997). A major breakthrough in the monitoring of *A. obscurus* and *A. lineatus* has been the identification of sex pheromones for these and other *Agriotes* species in the former USSR and Europe (e.g. Borg-Karlson *et al.* 1988). These pheromones were used successfully to survey the distribution of pest *Agriotes* species across the former USSR (Kudryavtsev *et al.* 1993). Based on this work, various pheromone blends were successfully tested for attractiveness to *A. obscurus* and *A. lineatus* in the lower Fraser Valley in 1999, and an effective prototype ground-based pheromone trap was concurrently developed (RSV unpublished data).

A simpler commercial version of the prototype pheromone trap was subsequently designed (currently known as the Vernon Beetle Trap, Phero Tech Inc., Delta, BC), and was used in delimitation surveys of *A. obscurus* and *A. lineatus* in BC and Washington State in 2000 and 2001 (Vernon *et al.* 2001). The trap design (details of which were not disclosed previously for proprietary reasons) has now been granted a U.S. Industrial Design patent (US Patent # Des. 465,254) and details of this trap can now be presented.

The trap (Fig. 1A) is constructed of durable polyvinyl chloride (PVC), and consists of two components formed from extrusion molds (Figs. 1B &C). The main component is an open ended box (Fig. 1B), the inside dimensions of which are 15.2 cm wide by 5 cm high. The other component is a ramp section (Fig. 1C), two of which are inserted into opposite ends of the open box. To assist beetles in climbing, the ramp section has 27, 0.2 mm high parallel ridges, spaced 2 mm apart, ending in a smooth downward curved section at the top. The ramp (width = 15.2 cm) slides easily into slots in the floor of the box component, with the ramp section angled upwards at 40° from the box floor. The top of the ramp is held at 3 mm from the top of the box component by indentations in the upper box corners and by 3 ridges extending downwards 3 mm into the box. The gap formed between the ramp top and ceiling of the box enables *Agriotes* click beetles to enter, while impeding entry by larger insects (e.g. large carabids) and other insectivores (e.g. voles). The ramp section also contains 2 parallel 7.0 mm deep protrusions, spaced 0.2 mm apart to allow for insertion of bubble cap pheromone release devices (Phero Tech Inc.). The length of the box component is 15.4 cm, such that when ramp sections are fully inserted into the open ends the opposing ramps are 1 cm apart at the curved apex. When deployed, the traps are placed at ground level, with the downward edges of the box section pushed into the soil, and the fully inserted entry ramps flush with and slightly covered by soil to provide unimpeded beetle entry.

When properly installed, the traps can

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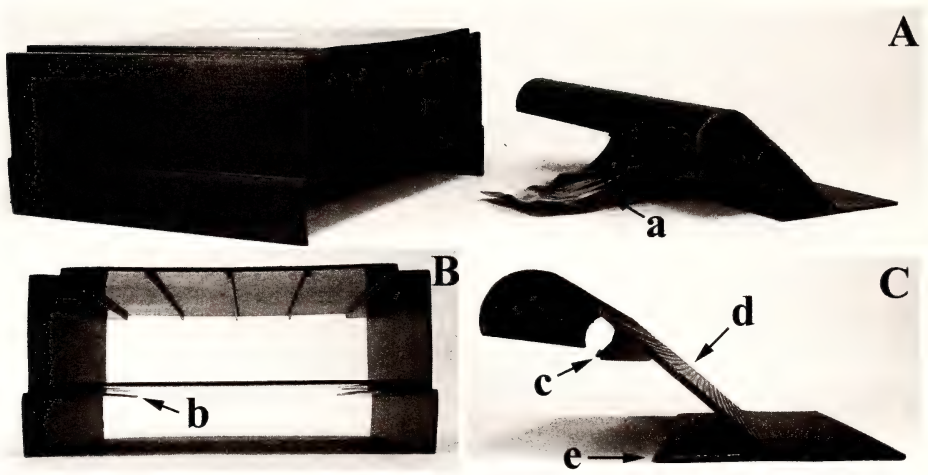

Figure 1. Photographs of a dismantled click beetle pheromone trap (A), showing the box section (B) and ramp section (C). Labelled details include: a) bubble-cap pheromone lure; b) floor slot for ramp insertion; c) lure clasp; d) ramp section with climbing ridges; and e) ramp insertion point.

often attract male *A. obscurus* and *A. lineatus* beetles within a few minutes. Trapping efficacy remains high throughout the adult generation of both species (Vernon *et al.* 2001), and other genera of click beetles are rarely captured. Traps with *A. lineatus* lures seldom catch *A. obscurus*, whereas *A. obscurus* traps will catch significant numbers of *A. lineatus*. Catch of *A. lineatus* in *A. obscurus* traps, however, can be almost eliminated if traps for both species are placed within 1.5 m of each other (RSV, unpublished data).

In a trial conducted in Agassiz B.C. comparing the relative efficacy of the new traps versus pitfall traps used in earlier elaterid surveys (Vernon and Päts 1997), the pheromone traps caught 54.3 *A. obscurus* per trap compared with only 1.6 *A. obscurus* caught per pitfall trap over a one month period in 2001. During this trial, escapes, and/or predation of click beetles caught in the pitfall traps was common, but was not observed in the pheromone traps. Some predation of click beetles by certain carabids (e.g. *Pterostichus melanarius* Illiger) will occur in pheromone traps left untended for 2-3 week periods late in the adult generations of *A. obscurus* and *A. lineatus* (RSV pers. obs.). When deployed in undisturbed areas and inspected routinely (10-14 days), however, the new pheromone traps provide an effective, convenient and inexpensive method for surveying and detecting *A. obscurus* and *A. lineatus* populations.

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SCIENTIFIC NOTE

Parasitoids of *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) in British Columbia

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ABSTRACT— Eggs of the western conifer seed bug, *Leptoglossus occidentalis* Heidemann, were parasitized in the field in British Columbia, Canada, by *Gryon pennsylvanicum* (Ashmead), *Anastatus pearsalli* Ashmead and an unidentified *Ooencyrtus* spp. Ashmead. *Leptoglossus occidentalis* represents a new host record for all three parasitoids. *Gryon pennsylvanicum* has not previously been reported in Canada.

The western conifer seed bug, *Leptoglossus occidentalis* Heidemann, feeds on several species of conifers (Hedlin *et al.* 1981), and can cause substantial yield losses in high-value seed orchards (Bates *et al.*, 2002; Strong *et al.* 2001). The generalist egg parasitoid, *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae), has recently been recovered from *L. occidentalis* egg masses in Italy (Camponogara *et al.* 2003) but little else is known about egg parasitoids of *L. occidentalis*

Members of the family Scelionidae are egg parasitoids of several economically-important hemipteran pests (Masner 1983). *Gryon pennsylvanicum* (Ashmead) is a polyphagous, solitary parasitoid of coreids, including *Anasa tristis* (De Geer) and several *Leptoglossus* species other than *occidentalis* (Masner 1983; Mitchell 1983; Yasuda 1990; Daane *et al.* 2001). *Anastatus pearsalli* Ashmead (Hymenoptera: Eupelmidae) is widely distributed throughout the nearctic, and parasitizes hosts from several orders and families including Coreidae (Burks 1979). Members of the genus *Ooencyrtus* (Hymenoptera: Encyrtidae) are also common egg parasitoids of a number of orders and families (Gordh 1979). We report on the occurrence of *G. pennsylvanicum*, *A. pearsalli* and *Ooencyrtus* sp. in B.C. in a previously undocumented host, *L. occidentalis*.

Leptoglossus occidentalis eggs were obtained by caging adult females on cone-bearing branches of lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann, at Kalamalka Seed Orchard, Vernon, B.C. (50.27 °N, 119.28 °W). In 2001, females were collected from orchard trees, and in 2002 they were obtained from an outdoor colony of overwintered insects maintained at Simon Fraser University. Eggs were laid in a single row along needles, and were collected by removing the entire needle. Ten egg masses, each bearing 10-13 eggs, were transferred to separate trees throughout the orchard on 5 July in 2001. In 2002, 24 egg masses were set out on trees on 30 June. Wire paper clips were used to fasten egg-bearing needles to foliage. Eggs were 0-7 d old at the time of transfer. After three weeks, all unhatched eggs were transferred to Petri dishes, maintained at room temperature in the laboratory and monitored for parasitoid emergence. Voucher parasitoid specimens were deposited in Canadian National Collection of Insects, Agriculture and Agri-Food Canada, Ottawa, Ontario.

In 2001, 32.7% of eggs were parasitized by an unidentified scelionid(s), prompting a more systematic study in the following year. In 2002, parasitoids

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emerged from ca. 29% of *L. occidentalis* eggs. *Gryon pennsylvanicum* was the predominant parasitoid, attacking 87% of parasitized eggs. The remaining parasitized eggs were parasitized by *Anastatus pearsalli* (8%) and an unidentified *Ooencyrtus* sp. (Hymenoptera: Encyrtidae) (4%). One parasitoid failed to complete its development and was not identified. *Leptoglossus occidentalis* has not previously been recorded as a host for any of these parasitoids.

Natural rates of parasitism of *L. occidentalis* eggs may vary at other times during the season. In addition, the use of eggs that were up to 7 d old may have affected the level of parasitism, because older eggs would have been acceptable to parasitoids for a shorter period of time. However, the relatively high level of parasitism observed in this study suggests that biological control with egg parasitoids could serve as a potential component of an integrated pest management program for *L. occidentalis* in B.C. seed orchards. Further study will be necessary to identify the full parasitic guild of this insect, its temporal synchronicity with *L. occidentalis*, and the density of wasps required to reduce seed bug populations. *Anastatus bifasciatus*, which was introduced into the eastern U.S. to control gypsy moth in the early 1900's (Crossman, 1925), may form part of the natural enemy complex of *L. occidentalis* in at least some regions of North America.

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SCIENTIFIC NOTE

Evaluation of the antiaggregation pheromone, 3-methylcyclohex-2-en-1-one (MCH), to protect live spruce from spruce beetle (Coleoptera: Scolytidae) infestation in southern Utah

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The spruce beetle, *Dendroctonus rufipennis* (Kirby), produces the antiaggregation pheromone 3-methylcyclohex-2-en-1-one (MCH) (Rudinsky *et al.* 1974). MCH has reduced the numbers of spruce beetles attracted to infested logs and synthetic semiochemical lures or reduced colonization rates throughout the beetles range (Kline *et al.* 1974, Rudinsky *et al.* 1974, Furniss *et al.* 1976, Dyer and Hall 1977, Lindgren *et al.* 1989). MCH has not prevented the infestation of live trees (Werner and Holsten 1995), with one exception. MCH in a novel formulation incorporating a microinfusion pump prevented the infestation of live spruce in Alaska in an area with a low spruce beetle population (Holsten *et al.* 2003). The objective of this study was to test MCH using commercially available diffusion releasers for protecting live trees from spruce beetle infestation in an area with a high spruce beetle population in southern Utah.

Study plots were located about 20 km east of Cedar City, Utah (lat. 37°38' N, long. 112°49' W) at elevations of 3,000 to 3,200 m, in a spruce beetle outbreak area. Circular, 1-ha plots were located in mixed stands of mature Engelmann spruce, *Picea engelmanii* Parry ex Engelm, and subalpine fir, *Abies lasiocarpa* Nutt. Two treatments (MCH application and untreated control) were replicated four times in paired plots. Pairs were about 100 m apart and replicates were 100-2,000 m apart. Plots were established on 24 and 25 June 1998 prior to spruce beetle flight. MCH-treated plots had 180 releasers stapled to the north side of trees and snags around the plot perimeter at a height of 2 m. Because of availability limitations, two different types of releasers were interspersed evenly with one another on each plot, 110 of the releasers were from IPM Technologies, Inc., Portland, Oregon, (release rate, 9 mg/day at 22 °C) and the other 70 releasers were from Phero Tech, Inc., Delta, British Columbia, Canada, (release rate, 7 mg/day at 25 °C). A multiple-funnel trap baited with a lure containing frontalin and α-pinene in polyvinyl chloride formulations each releasing 0.8 mg/day at 25 °C was placed at the center of each plot to monitor beetle activity. Traps were emptied on 2 and 7 July 1998, and were removed when successful beetle colonization on trees within the plots was first observed. The basal area of all trees ≥20 cm diameter at breast height (dbh) was measured at 30 m from the plot center in the four cardinal directions and recorded by species. Percent spruce basal area was calculated. Plots were surveyed on 17 September 1998 after beetle flight had ended to determine the dbh and infestation status of all spruce ≥20 cm dbh. Trees were classified as mass-attacked or unattacked based on the presence or absence of large amounts of boring dust on the lower third of the bole. Percentage of spruce trees ≥20 cm dbh that were mass-attacked was calculated for each plot.

Paired *t*-tests were used to test for treatment differences in the total numbers

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of spruce beetles caught in the traps and in the tree and stand data. Percentages of spruce trees mass-attacked were arcsin square root transformed. Nontransformed means are reported.

There was no significant difference in spruce beetle catches between MCH-treated and untreated plots (P = 0.7430). The average (± SE) number of spruce beetles caught in traps on MCH-treated plots was 546 ± 293 and the average caught in traps on controls was 473 ± 245. As expected, there were no significant differences between treatments for basal area (P = 0.2113), percent of total basal area (P = 0.9409), tree density (P = 0.6715), or dbh (P = 0.4592), since plots were selected to be similar with respect to stand structure and composition. Furthermore, the percent of spruce ≥20 cm dbh that were mass-attacked by the spruce beetle was not significantly different on MCH-treated (52.7 ± 20.3%) and untreated plots (68.3 ± 15.3%) (P = 0.4262). The majority, if not all, of the colonized trees were heavily infested.

The application rate of MCH used in this study was more than twice the current recommended dose for the Douglas-fir beetle (Ross *et al.* 2001). Despite the high application rate, MCH was not effective in preventing host-tree infestation by the high-density spruce beetle population. The lack of a significant effect of MCH might have been related to release rates of the compound under field conditions or to the lack of a behavioral response of the species to the compound. A recent study demonstrated that spruce beetle host selection behavior changes with population density (Wallin and Raffa 2004) and this could explain the different responses to MCH that have been observed in field studies. Further study will be needed to determine the conditions under which MCH might be operationally feasible for protecting live spruce.

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SCIENTIFIC NOTE

New waterboatmen records for Western Canada (Hemiptera: Corixidae)

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ABSTRACT— *Trichocorixa verticalis* (Fieber) is reported for the first time from the mainland of British Columbia and the subspecific assignment is discussed. Based on specimens in the Spencer Entomological Museum, one provincial record and one territorial record are added to the recent checklist of Canadian Hemiptera.

During recent collecting trips in the Lower Mainland of British Columbia (BC), Canada, we found a population (both immatures and adults) of *Trichocorixa verticalis* (Fieber). Previously, *T. verticalis* was known in BC only from three island localities: two on Vancouver Island and one on Thetis Island (Scudder 1977). A fourth island record can be added to these: Prevost Island, salt marsh pond, 10.vii.1986, J.D. Reynolds, 1 male, 2 females, Spencer Entomological Museum.

Outside of BC, *Trichocorixa verticalis* has a widespread distribution. Two subspecies occur on each of the east and west coasts of North America with a fifth subspecies broadly distributed through the central plains (Sailer 1976). The subspecies *T. v. californica* Sailer was described based on specimens from central California (Sailer 1976) and its known distribution was subsequently extended north through Oregon and Washington (Stonedahl & Lattin 1986). All previous BC records have been attributed to this subspecies (Scudder 1977). *Trichocorixa verticalis californica* is listed as potentially rare or endangered in British Columbia (Scudder 1994) and is considered a species of special concern in the Georgia Depression Region (Scudder 1996).

The mainland *Trichocorixa* specimens were collected in Delta, south of Vancouver, from a ditch on the north side of Deltaport Way in early October 2003 and again in late May 2004. The ditch runs inland from the coast and connects at its coastal end to a second ditch that runs parallel to and just inland of the coastal dike. In the October survey, semi-quantitative data were collected between 1.3 km and 3.7 km inland from the dike. The density of *T. verticalis* was found to drop sharply beyond 1.8 km and no specimens were collected more than 2.2 km from the dike. In May, we extended the surveyed area to the coastal dike; *T. verticalis* was found at all sites between 0 and 1.3 km inland.

While the assignment of the Delta specimens to *T. verticalis* was straightforward, the subspecific assignment was not. In his key to subspecies of *T. verticalis*, Sailer (1976) deals separately with female and male specimens. Based on those keys, our female specimens are *T. v. californica* but our male specimens are *T. v. verticalis* (Fieber). The range for *T. v. verticalis* extends along the coast from Maine to Mexico and includes the West Indies (Sailer 1976). In Sailer's key, a critical character for males is the ratio of the length of the closest separation of the eyes (IO) to the length of the hind margin of the eye (L_e). In *T. v. californica* the ratio is greater than or equal to one; the ratio is less than one in *T. v. verticalis*. In our specimens, IO:L_e is less than one. In order to check Sailer's key, this ratio was measured for two male paratypes of *T. v. californica* (CA, San Mateo Co., Moss Beach, 4.vii.1929, R.L. Usinger) and for two male specimens of *T. v. verticalis* (Bermuda Island, Spittal Pond, 10.viii.1940, G. Kelly); all four specimens are in the Essig Museum at the University

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of California Berkeley. These four specimens fulfill Sailer's criteria.

In a further effort to identify our specimens, we examined the left and right claspers from one of the male specimens. Comparison of these claspers with the drawings for the various species of *Trichocorixa* in Sailer (1976) showed the best agreement between our specimen and *T. verticalis*. However, within the subspecies of *T. verticalis*, the claspers of our specimen are in poorest agreement with those shown for *T. v. californica*; they are in much better agreement with those of the three more easterly subspecies.

We rechecked the identification of all of the BC *Trichocorixa* specimens in the Spencer Entomological Museum (three localities with a total of two males, five females and one immature). The five female specimens key out to *T. v. californica*. One of the males is teneral and is partially collapsed; it cannot be run through the key. The second male is damaged but the ratio of IO: L_e can be measured and is less than one, in agreement with the Delta specimens. Thus at least one of the male *Trichocorixa* specimens previously collected in BC also does not fit Sailer's description of *T. v. californica*. It is unclear whether the definition of *T. v. californica* needs to be broadened to account for the geographical variation represented by the BC specimens or if the BC specimens belong to a different subspecies. A re-examination of the whole question of subspecific designations for *T. verticalis* is needed. In view of the uncertainty in assigning subspecies to the BC specimens, we prefer to leave the determination as simply *T. verticalis*.

In addition to *T. verticalis*, we collected four other species of corixids from the ditch in Delta: *Cenocorixa blaisdelli* (Hungerford), *Corisella inscripta* (Uhler), *Hesperocorixa atopodonta* (Hungerford) and *Sigara omani* (Hungerford). *Cenocorixa blaisdelli* is listed as potentially rare or endangered in British Columbia (Scudder 1994) and *Corisella inscripta* is listed as rare or very local in occurrence in Canada (Maw *et al.* 2000). These records highlight the importance of surveying these often overlooked habitats. Voucher specimens for all of these records are in the Spencer Entomological Museum.

Examination of the corixids in the collection at UBC showed that two entries were omitted from the recent Checklist of Hemiptera of Canada and Alaska (Maw *et al.* 2000), one each for BC and NT, based on the following records: ***Hesperocorixa minorella* (Hungerford)**: BC, Banks Is., Kooryet Bay, sphagnum bog, 11.viii.1986, G.G.E. Scudder, 1 male, 4 females; BC, Diana L., sphagnum bog, 14.viii.1986, G.G.E. Scudder, 2 males. ***Sigara lineata* (Forster)**: NT, Hay R., Mackenzie Hwy., 20.vii.1961, T.G. Northcote, 1 male, 2 females.

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SCIENTIFIC NOTE

Polistes dominulus (Christ) (Hymenoptera: Vespidae: Polistinae) in British Columbia: first collection records of an invasive European paper wasp in Canada

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Two species of the cosmopolitan genus *Polistes* (paper wasps), *P. fuscatus* (Fabricius) and *P. aurifer* Saussure, are native to British Columbia (Carpenter 1996), although some entomologists consider *P. aurifer* a subspecies of *P. fuscatus* (Kenner 2002). These wasps are frequently confused with yellowjacket species (Vespinae) but are easily distinguished by their thread waist, their habit of trailing their legs in flight, and their nests of exposed cells. They frequently nest on man-made structures.

The European paper wasp, *P. dominulus* (Christ) (Fig. 1), native to Europe, Asia and North Africa, has been introduced into the USA, Australia and Chile (Carpenter 1996). It was first recorded in the USA from Massachusetts in the late 1970s (Eickwort 1978) and its range has since expanded south and west, covering most of the northeastern states (Judd and Carpenter 1996, Pickett and Wenzel 2000, Gamboa *et al.* 2004, Johnson and Starks 2004). It has also moved north to Kingston (sighted in 2002) and Sandfield (2004), Ontario (H. Goulet, Agriculture and Agri-food Canada, pers. comm.), but apparently no specimens have been collected in eastern Canada. *Polistes dominulus* has recently appeared in the western USA from Washington to California and east to Colorado (Landolt and Antonelli 1999, Pickett 2003). It is not clear if these populations are the result of new introductions, possibly from Asia, or of a western expansion of eastern introductions (Johnson and Starks 2004).

In British Columbia, *P. dominulus* was first recorded in late August 2003, when D. Manastryski (3808 Rowland Dr. Victoria, BC) photographed a nest (identified by H. Goulet) in Saanich. No specimens were collected, but the photograph was published on the back cover of the Entomological Society of Canada 2003 meeting program. On 5 September 2004, while walking along the shore of Shuswap Lake in Salmon Arm, the first author found a pre-hibernation cluster of about 25 unusual wasps under an overhang on a wooden signpost. One was collected and identified as *P. dominulus*. Art Borkent (Royal BC Museum, Victoria, BC) collected 10 wasps from the same cluster on 20 September 2004. There was no nest or nest-building activity; this late-season aggregation behaviour has been previously reported for the species (Landolt and Antonelli 1999).

On 30 September 2004, a *P. dominulus* nest was collected from a nail protruding from the eaves of a house in Saanich, BC. The nest had three female wasps on its surface (Fig. 1) and 17 between the eaves and the back of the nest. The wasps may have congregated for warmth; the temperature was 7 °C (2130 PDT). The nest held 206 cells, six of which contained larvae in various stages of development. A few other cells were less than a third filled with nectar. This cell number falls well within the range documented for the species by Pickett and Wenzel (2000). None of the specimens collected at either site showed signs of Strepsiptera parasitism. Voucher specimens from both localities are deposited at the Royal BC Museum, Victoria, BC, and the Canadian National Collection of Insects, Ottawa, Ontario.

Polistes dominulus is distinguished from the native *P. aurifer* and *P. fuscatus*

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by the yellow patterns on the thorax (Fig. 2). *Polistes dominulus* individuals are usually the size of yellowjacket wasps, making them smaller than native species.

Although *P. dominulus* is clearly expanding its range, the effect of this invasion on native species is not as easily determined (Pickett and Wenzel 2000, Johnson and Starks 2004). The recent appearance of *P. dominulus* in BC provides an opportunity to measure its effect on native *Polistes* populations. Probably it will increase in abundance within its new BC range, resulting in many more observations in and around human dwellings, where it prefers to nest.

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Figure 1. Females of *Polistes dominulus* at a nest in Saanich, BC, 30 September 2004.

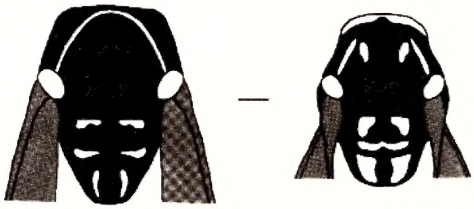

Figure 2. Diagrammatic representation of the thorax of *Polistes aurifer* or *P. fuscatus* (left) and *P. dominulus* (right), showing differences in size and markings (white areas = yellow). Gray areas represent wings. Scale line = 1 mm.

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